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Letter

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Identification of "Preferred" Human Kinase Inhibitors for Sleeping Sickness Lead Discovery. Are Some Kinases Better than Others for Inhibitor Repurposing?

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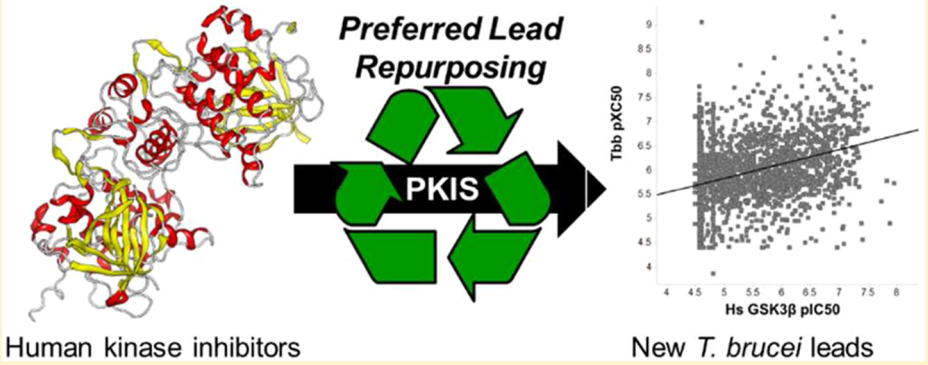
Supporting Information



ABSTRACT: A kinase-targeting cell-based high-throughput screen (HTS) against *Trypanosoma brucei* was recently reported, and this screening set included the Published Kinase Inhibitor Set (PKIS). From the PKIS was identified 53 compounds with $pEC_{50} \geq 6$. Utilizing the published data available for the PKIS, a statistical analysis of these active antiparasitic compounds was performed, allowing identification of a set of human kinases having inhibitors that show a high likelihood for blocking *T. brucei* cellular proliferation in vitro. This observation was confirmed by testing other established inhibitors of these human kinases and by mining past screening campaigns at GlaxoSmithKline. Overall, although the parasite targets of action are not known, inhibitors of this set of human kinases displayed an enhanced hit rate relative to a random kinase-targeting HTS campaign, suggesting that repurposing efforts should focus primarily on inhibitors of these specific human kinases. We therefore term this statistical analysis-driven approach *"preferred lead repurposing"*.

KEYWORDS: *preferred lead repurposing, Trypanosoma brucei, Trypanosoma cruzi, Leishmania major, Plasmodium falciparum, Published Kinase Inhibitor Set*

Neglected tropical diseases (NTDs) are a collection of infectious diseases that contribute to a significant level of morbidity and mortality, particularly in the developing world. For example, human African trypanosomiasis (HAT) is caused by the insect-borne protozoan pathogen *Trypanosoma brucei*, and the resulting infection results in 1.6 million disability-adjusted life years.[1] Although the disease burden caused by these NTDs is high, there is little financial incentive to engage in the costly process for the discovery and development of drugs, which leaves a current pharmacopeia of mixed efficacy,

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safety, and convenience. To wit, one of the front-line treatments for HAT is melarsoprol, which is a brain-penetrant organoarsenic agent that itself has at least a 5% mortality rate.[2]

As a result, we and others have been advancing efforts to repurpose classes of investigational drug agents as new leads for HAT and other NTDs. Termed "target repurposing"[3] or "piggy-back"[4] drug discovery, these efforts entail assessment of established inhibitors of human enzymes that are homologous to essential parasite enzymes and pursuit of these and related analogues as a launching point for new antiparasitic agents. We have found that kinase inhibitors represent a particularly fertile area for application of target repurposing,[5−9] and our pursuit of this family of inhibitors led to a high-throughput kinase-targeted inhibitor screen against *T. brucei*. In that 42444 compound screening campaign we identified 797 potent ($pEC_{50} \geq 6$) and selective (>100×) inhibitors of the bloodstream form of the parasite.[10]

Included in the high-throughput screening (HTS) set was the Published Kinase Inhibitor Set (PKIS) released by GlaxoSmithKline (GSK), which consists of a set of 369 kinase inhibitor compounds with inhibitory data available against 224 human kinases at two concentrations (0.1 and 1 μM), providing information regarding each compound's human kinase selectivity.[11] Publicly available in screening plates upon request from GSK, this inhibitor set has been utilized by a number of research groups outside GSK, culminating in a number of papers describing exploration of a wide range of programs of relevance to human health.[12]

During the analysis of our HTS results, we noted that there were particular families of kinase inhibitors present in the PKIS collection that showed high potency against *T. brucei*, with good-to-excellent selectivity over host cells (HepG2, see Table S1 in the Supporting Information). This observation led us to ask whether a more rigorous analysis of the PKIS screening hits might uncover more information about which human kinases may share inhibitor sensitivity similar to that of *T. brucei* cells in vitro.

Although target repurposing approaches are most often launched by a bioinformatic analysis that matches human-to-parasite based on *target protein sequence similarity*, we hypothesized that an analysis of the PKIS may lead to an ability to match on the basis of similarity of *inhibitor sensitivity*. Such an analysis could allow a more focused approach to repurposing established kinase inhibitor chemotypes, even in the absence of information about specific parasite target inhibition. We felt that this approach could be valuable given previous work that demonstrated that repurposing chemical matter from human homologues can be successful and be causing their effect, at least in part, by inhibition of the parasite homologue, such as for Aurora kinase,[13] or GSK3β.[14] However, because much is unknown about the function of many *T. brucei* kinases, multiple targets may be contributing to the antiproliferative effects of a given kinase inhibitor, as was observed for DDD34425, a potent inhibitor of *T. brucei* PK50.[14] Furthermore, although *T. brucei* does not express canonical protein tyrosine kinases,[15] human tyrosine kinase inhibitors have previously been successfully repurposed to launch lead discovery programs.[7,16]

In light of these observations, we wished to utilize the unique juxtaposition of *T. brucei* and human kinase activity data to identify whether there was a significant probability that inhibitors of particular human kinases were more active against *T. brucei* when compared to a wider set of kinase inhibitors and whether these might provide a good starting point to launch new lead discovery efforts, even in the absence of parasite target information.

To accomplish this, we systematically analyzed the PKIS data to find statistical associations between human kinase inhibition and *T. brucei* inhibition. Before that, the 369 compounds included in the PKIS were assessed for completeness of data: despite meeting the primary HTS cutoff, four compounds had incomplete EC_{50} data against *T. brucei* cells, and/or HepG2 cellular selectivity data. Thus, the overall compound set was reduced to 365.

Compounds were binned on the basis of their *T. brucei* pEC_{50} (Table 1). For the human kinase inhibitory analysis, each compound was assigned a score on the basis of whether the compound showed ≥70% inhibition (scored 1) or <70% inhibition (scored 0) at 0.1 μM. The total number of inhibiting compounds was calculated for each kinase. In general, these calculations gave us the number of compounds with a *T. brucei* pEC_{50} in a particular range (e.g., $pEC_{50} \geq 6$) that displayed an inhibitory activity against the selected human kinase higher than or equal to the selected cutoff of 70% inhibition at 0.1 μM. This process was repeated for higher cutoff values of kinase inhibition (≥80 and ≥90% inhibition at 0.1 μM) and for several *T. brucei* pEC_{50} ranges. A total of 2016 2×2 contingency tables were constructed. Fold enrichments were calculated, and a chi-squared contingency table test was performed to evaluate the statistical significance of the enrichments.[17,18] A contingency table was considered significant when its chi-squared test p value was <0.01.

Table 1. Inhibitors Binned on the Basis of *T. brucei* Proliferation Inhibition

pEC_{50} range	no. of compounds
<4	253
≥4	112
≥6	53
≥7	13

The contingency tables for each kinase were then sorted by their fold enrichment (see Methods for definition). In the interest of using the same cutoff value as used in the original *T. brucei* HTS, we elected to utilize the $pEC_{50} \geq 6$ cutoff to denote "active" parasite proliferation inhibitors, which provided a large enough sample to provide a statistically valid analysis. The highest-scored kinases (by fold enrichment) and their respective p values are reported in Table 2.

On the basis of these results, we note that the most highly scored kinases belong to the human CMGC kinase family, which includes DYRK, CDK, GSK-3, and HIPK. Lower fold enrichment values have been found for tyrosine kinases (TK) (LCK, ROS, LTK, IGF1R, ALK, PYK2, INSR, and ABL mutant variants) and tyrosine-kinase-like kinases (TKL) (LRRK2 and its mutant variant LRRK2-G2019S). A graphical representation of the kinase enrichment scores, grouped by kinase family, is reported in Figure 1.

The translation of these observations to prospective application would be desirable. Thus, on the basis of the statistical analysis performed, we selected from commercial vendors 26 established inhibitors of the "preferred" human kinases (Tables S2 and S3, Supporting Information). To assess the structural similarity of the commercial inhibitors with the PKIS compounds, the Tanimoto coefficients between all of the

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Table 2. Highly Scored Human Kinases for Active *T. brucei* Inhibitors Defined as $pEC_{50} \geq 6$, Grouped by Human Kinase Percent Inhibition Cutoffs[a]

			70% inhibition cutoff		80% inhibition cutoff		90% inhibition cutoff	
kinase	family	N[b] (active vs *T. brucei*)	enrichment	p value	enrichment	p value	enrichment	p value
DYRK1B	CMGC	6 (6)	41.20	6.06×10^{-8}	35.32	1.19×10^{-6}		
DYRK1A	CMGC	6 (6)	41.20	6.06×10^{-8}	23.54	3.92×10^{-4}		
ARK5	CAMK	4 (4)	29.43	2.24×10^{-5}				
CDK1/cyclinB	CMGC	3 (3)	23.54	3.92×10^{-4}				
CDK3/cyclinE	CMGC	3 (3)	23.54	3.92×10^{-4}				
HIPK1	CMGC	6 (5)	17.66	1.07×10^{-5}	17.66	1.07×10^{-5}		
CDK5/p35	CMGC	2 (2)	17.66	6.17×10^{-3}				
NEK9	other	2 (2)	17.66	6.17×10^{-3}				
HIPK4	CMGC	2 (2)	17.66	6.17×10^{-3}				
LCK	TK	2 (2)	17.66	6.17×10^{-3}				
GSK3α	CMGC	13 (9)	11.77	4.26×10^{-8}	11.77	5.89×10^{-5}		
CLK2	CMGC	7 (5)	11.77	5.89×10^{-5}	11.77	5.89×10^{-5}	17.66	1.07×10^{-5}
TSSK1	CAMK	7 (5)	11.77	5.89×10^{-5}	11.77	5.89×10^{-5}	17.66	6.17×10^{-3}
LRRK2	TKL	7 (5)	11.77	5.89×10^{-5}	9.81	7.05×10^{-4}		
ROS	TK	7 (5)	11.77	5.89×10^{-5}				
GSK3β	CMGC	12 (8)	10.59	5.65×10^{-7}	9.81	7.05×10^{-4}		
LTK	TK	9 (6)	10.30	2.04×10^{-5}	7.35	2.22×10^{-3}	9.81	7.05×10^{-4}
CDK2/cyclinE	CMGC	9 (6)	10.30	2.04×10^{-5}	17.66	6.17×10^{-3}		
TTK	other	6 (4)	9.81	7.05×10^{-4}				
IRR	TK	8 (5)	8.83	2.29×10^{-4}	7.84	7.14×10^{-3}	7.84	7.14×10^{-3}
CDK2/cyclinA	CMGC	13 (8)	8.83	2.23×10^{-6}	8.24	7.37×10^{-5}		
IGF1R	TK	10 (6)	8.24	7.37×10^{-5}	8.24	7.37×10^{-5}	10.30	2.04×10^{-5}
ALK	TK	10 (6)	8.24	7.37×10^{-5}	7.06	6.95×10^{-4}		
LRRK2-G2019S	TKL	5 (3)	7.84	7.14×10^{-3}	11.77	2.13×10^{-3}		
PYK2	TK	9 (5)	7.06	6.95×10^{-4}				
INSR	TK	11 (6)	6.86	2.18×10^{-4}	6.86	2.18×10^{-4}	7.06	6.95×10^{-4}
PLK1−002	other	16 (8)	5.88	5.37×10^{-5}	8.83	2.23×10^{-6}	11.77	1.61×10^{-6}
ABL-Q252H	TK	5 (3)			7.84	7.14×10^{-3}		
ABL-M351T	TK	3 (2)			7.84	7.14×10^{-3}		

[a]Kinases with missing data do not display a statistically significant enrichment at the respective percent inhibition cutoff. [b]N, number of inhibitors from the PKIS that show ≥70% inhibition of the preferred kinase at 0.1 μM concentration; the number in parentheses represents the number of these inhibitors with *T. brucei* $pEC_{50} \geq 6$.

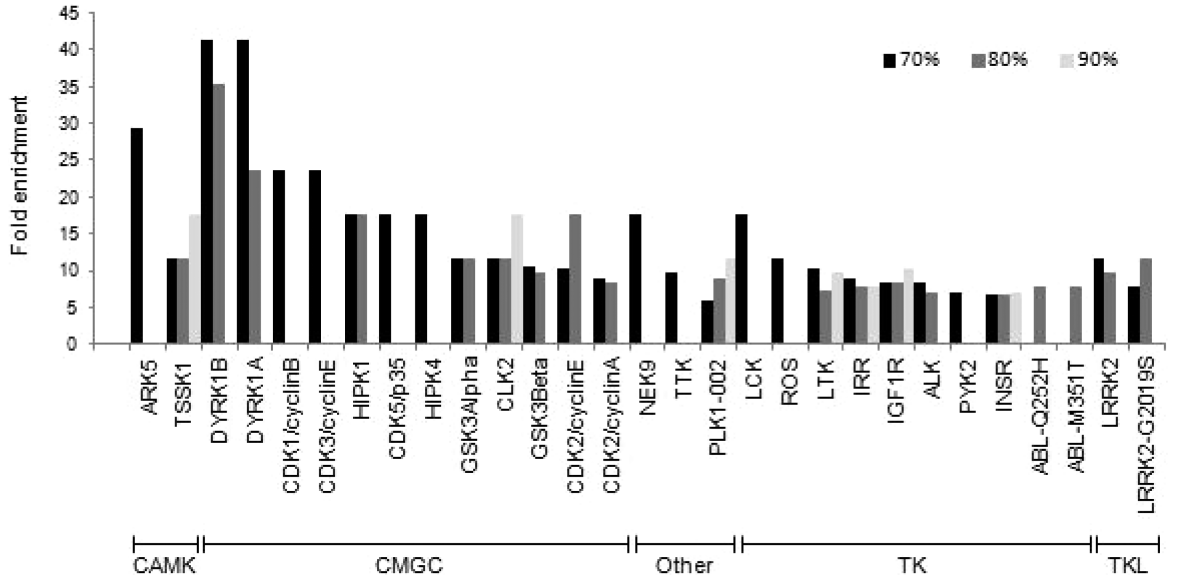

Figure 1. Fold enrichment of human kinases, grouped by family, using three human kinase percent inhibition cutoffs (70, 80, and 90%). Kinases missing histogram bars do not display a statistically significant enrichment at the respective percent inhibition cutoff.

former were calculated against all of the latter. We wished to ensure that any enhancement to the hit rate would not be the result of testing close analogues of the original PKIS compounds. The similarity assessment results are shown in

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Figure 2 in the form of box plots that show the range of structural similarity between the purchased compounds and

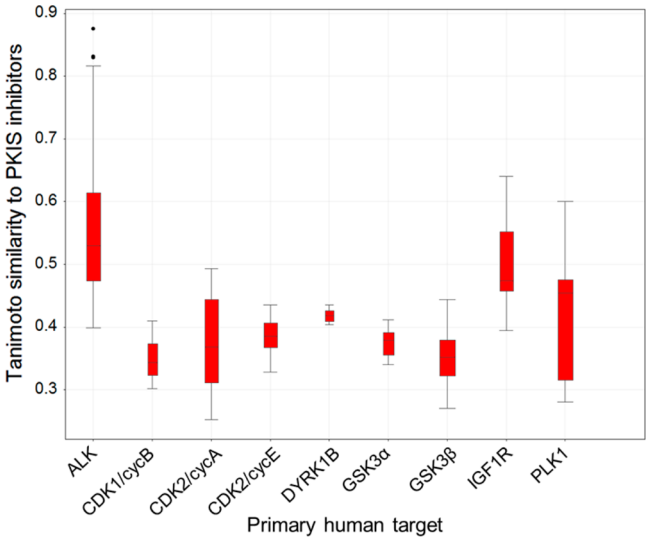

Figure 2. Box plots showing Tanimoto scores of the purchased kinase inhibitors shown in Table 3 to members of the PKIS that inhibit the same primary human target. The primary human target is defined as the highest percent inhibition at 0.1 μM drug concentration.

PKIS inhibitors, grouped by the preferred kinase. (These data are also found in Table S4 of the Supporting Information.)

Confident in the structural diversity of these purchased compounds over the original PKIS hits, we assessed them using the same *T. brucei* assay that was performed in the HTS campaign. The results of these screening experiments are shown in Table 3; 13 compounds showed a growth inhibition $pEC_{50} \geq 6$. This result represents an overall 50% hit rate (13/26) (compared to 4.2% for the overall HTS, which was performed on a random set of kinase-targeting inhibitors (1797 compounds with $pEC_{50} \geq 6$, of 42444)). The *T. brucei* hit rate of the PKIS collection was 14.3% (53/369).

For example, Figure 3 shows the progression from GSK1173862A, an "active" ALK inhibitor in the PKIS, to four purchased ALK inhibitors. We note that two of the purchased inhibitors of human ALK (crizotinib and NVP-TAE684) are potent *T. brucei* proliferation inhibitors, despite their structural uniqueness compared to the original PKIS hit chemotype.

We wished to test the significance of the observed enrichments using a wider set of investigational inhibitors of "preferred" kinases. We performed a retrospective analysis of HTS hit compounds that had previously emerged from internal GSK kinase inhibitor discovery programs. The expectation would be that if we were to select compounds from the HTS that were originally designed for one of the "preferred" human kinases, we should see an enhancement of hit rate against *T. brucei* when compared to inhibitors that were originally designed for other human kinases. The GSK database was searched to find screening hits active against the preferred kinases. After the removal of any PKIS compounds and their analogues included in this data set, nine human kinase screens were selected where at least 100 molecules were shared with the *T. brucei* screen. Contingency tables were built by comparing "actives/inactives" of *T. brucei* versus "actives/inactives" of the tested kinase, where "actives" are defined as having ≥80% inhibition in single-concentration assays. Chi-squared contingency table tests were performed and the corresponding p values determined as described above. Of these kinases analyzed in this way (ALK, ARK5, CDK2/cyclinA, GSK3β, DYRK1A, IGF1R, LCK, LRRK2, and PLK1), all showed conclusive evidence of significance in the chi-square test ($p < 0.01$ and enrichments > 1; Figure 4).

In addition, we checked that "non-preferred" kinases from our analysis did not show statistical association with *T. brucei* inhibition by using non-PKIS compounds in retrospective GSK screens (Figure S4 in the Supporting Information).

These results, obtained with compounds structurally different from the PKIS and from the commercial set of inhibitors, further demonstrate that these particular human kinases are "preferred" in terms of their susceptibility to inhibitors that also show a high probability of anti-*T. brucei* activity.

Finally, we were interested to know whether these "preferred" *T. brucei* proliferation inhibitors would have a similar activity against other protozoan parasites. We reasoned that this would be possible because the three related kinetoplastid parasites, *T. brucei*, *T. cruzi*, and *Leishmania major*, have highly similar kinomes.[15] Thus, in the interest of trying to ascertain cross-parasite relevance of these "preferred" kinase inhibitors, we also assessed these compounds against *T. cruzi* intracellular amastigotes (Tulahuen strain), as well as against *L. major* promastigotes and intracellular amastigotes. In addition, we tested these inhibitors against the malaria-causing protozoan *Plasmodium falciparum* (D6 strain). Little activity was observed, indicating that these inhibitors are apparently "preferred" only as *T. brucei* growth inhibitors (see Table 3 and Figure S2 in the Supporting Information).

In summary, by testing the PKIS against *T. brucei* cells, we were able to identify kinases having inhibitors that are predisposed to be active against *T. brucei* cells. By performing a prospective analysis using structurally unique, commercially available inhibitors of these preferred kinases, and a retrospective analysis of historical GSK kinase HTS data, we confirmed that, in fact, inhibitors of these kinases are excellent starting points for launching new *T. brucei* drug discovery efforts. In contrast to using a human/parasite target homology-based approach for compound selection (also known as *target repurposing*), the utilization of such a "preferred" kinase analysis represents a method for a ligand-centric hit identification process that we term "*preferred lead repurposing*". Although we have presented evidence of such an approach working for hit identification for *T. brucei* drug discovery, the hits identified are not necessarily promising hits for other protozoan parasites. In due course, as PKIS screening results are reported for other pathogens such as *T. cruzi*, *L. major*, or *P. falciparum*, we look forward to identification of "preferred kinases" that can help focus lead repurposing efforts for these pathogens, as well. Finally, we note that several of the potent and nontoxic *T. brucei* proliferation inhibitors that we have identified in this work are compounds that have advanced into human clinical trials for a variety of indications. Encouraged by this, we have launched a program to further evaluate some of these compounds for direct repurposing against HAT and have initiated systematic medicinal chemistry programs for others to optimize potency and properties to be consistent with the targeted profile for new HAT therapeutics.

We note that some compounds may not be well suited for antiparasitic agents by virtue of their potent activity against human kinases. However, given that some of these kinase

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Table 3. Inhibitors of Preferred Human Kinase Purchased and Tested against Protozoan Pathogens and Host Cells

				T. brucei	*Leishmania*		*T. cruzi*	*P. falciparum*			host cell tox	
no.	compd	compd name	human kinase (pIC_{50})	pEC_{50} (within 46%)	Promast pEC_{50}	Amast pEC_{50}	pEC_{50} (μM) (within 21%)	D6 pEC_{50}	W2 pEC_{50}	C235 pEC_{50}	3T3 pTC_{50}	HepG2 pTC_{50}
1	NEU-838	crizotinib	ALK (7.6)	6.4	6.2	5.8	5.1	6.1	7.4	7.0	4.8	5.4
2	NEU-844	TAE684	ALK (8.5)	7.0	6.5	5.9	5.3	6.3	6.4	6.3	4.6	5.3
3	NEU-893	GSK1838705A	ALK (9.3)	5.7	<4.6	<4.8	5.0	5.6	5.8	5.8	4.8	5.1
4	NEU-967	NVP-AEW5414	IGF-1R (6.8)	5.7	7.0	5.4	5.4	5.9	6.0	5.9	4.8	5.5
5	NEU-968	BMS-2652465	CDK1-cyclinB (8.2)	6.3	6.6	<4.8	<4.3	4.8	5.0	4.9	<4	<4.4
6	NEU-969	PHA-8481256	CDK2-cyclinA (7.3)	7.6	6.5	5.3	7.4	5.2	5.1	5.2	<4	5.0
7	NEU-970	GSK1904529A7	IGF-1R (7.6)	5.9	<4.9	5.5	<4.3	6.8	7.0	6.9	<4	<4.4
8	NEU-971	JNJ-77066218	CDK2-cyclinE (8.5)	6.5	<4.6	<4.8	<4.3	5.7	6.0	5.9	<4	5.2
9	NEU-973	ON-019109	PLK1 (8.0)	<5.5	<4.6	<4.8	<4.3	<4.7	<4.7	<4.7	<4	<4.4
10	NEU-974	CH542480210	ALK (8.7)	<5.5	6.9	<4.8	<4.3	5.4	<4.7	<4.7	<4	<4.4
11	NEU-975	dinaciclib	CDK2 (9.0) P35/CDK5 (9.0)	7.4	5.8	5.6	7.0	5.9	6.1	6.5	<4	<4.4
12	NEU-976	linsitinib	IGF1R (7.5)	<5.5	5.1	<4.8	5.0	6.3	5.7	6.3	<4	4.5
13	NEU-977	roscovitine 13	P35/CDK5 (9.0)	<5.5	5.2	<4.8	<4.3	6.1	5.7	5.7	<4	4.5
14	NEU-978	CHIR-9801414	GSK3α/β (9.2)	<5.5	5.2	<4.8	<4.3	<4.7	<4.7	<4.7	<4	4.8
15	NEU-979	volasertib	PLK1 (9.1)	<5.5	5.8	5.1	4.9	6.8	6.8	6.8	4.6	5.5
16	NEU-980	BI 253616	PLK1 (9.1)	6.2	5.4	5.1	4.7	7.7	8.0	7.9	4.5	5.6
17	NEU-982	AT751918	p35/CDK5 (7.9)	6.5	<4.6	<4.8	5.9	5.9	6.1	6.5	<4	<4.4
18	NEU-984	SNS-03219, 20	CDK9/cyclinT (8.4)	6.9	<4.6	<4.8	< 4.3	5.3	5.5	5.7	<4	<4.4
19	NEU-985	AZD543821	CDK2/cyclinE (8.2)	7.5	6.4	5.6	5.8	5.9	6.0	5.9	<4	4.9
20	NEU-986	SB 41528622	GSK-3α (7.1)	6.1	<4.6	<4.8	<4.3	4.8	4.6	4.6	4.2	<4.4
21	NEU-987	flavopiridol	CDK1/2/4/6 (7.4)	6.8	6.4	5.5	4.9	5.7	5.7	5.8	< 4	< 4.4
22	NEU-989	TWS11925	GSK-3β (7.5)	6.0	4.7	<4.8	<4.3	5.0	4.7	5.1	4.4	<4.4
23	NEU-990	SB 21676322	GSK-3α (7.5)	<5.5	5.6	<4.8	<4.3	<4.7	<4.7	<4.7	<4	<4.4
24	NEU-991	tideglusib	GSK-3β (7.2)	<5.5	6.5	<4.8	4.7	4.6	4.6	4.7	4.1	<4.4
25	NEU-1007	PHA-79388727	p25/CDK5 (8.3)	5.7	<4.6	<4.8	<4.3	5.7	5.6	5.6	<4	<4.4
26	NEU-1049	harmine	DYRK1B (7.6)	<5.5	5.9	5.9	4.8	<4.7	<4.7	<4.7	<4	<5.1

inhibitors are tolerated in humans for extended dosing regimens and that antiparasitic therapies would likely require a much shorter treatment period, we posit that in some cases exquisite selectivity for *T. brucei* cells versus over host kinases may not be required. On the other hand, it would seem prudent to minimize such host kinase activities, which would be the goal of further medicinal chemistry optimization. We propose that focusing on inhibitors of "preferred" human kinases provides a strong starting point for such optimization programs.

METHODS

Biological Assays. The *T. brucei* and HepG2 cell assays were performed as previously described,[10] as were the *P. falciparum*, *L. major*, and *T. cruzi* assays.[8] The HepG2 cells were

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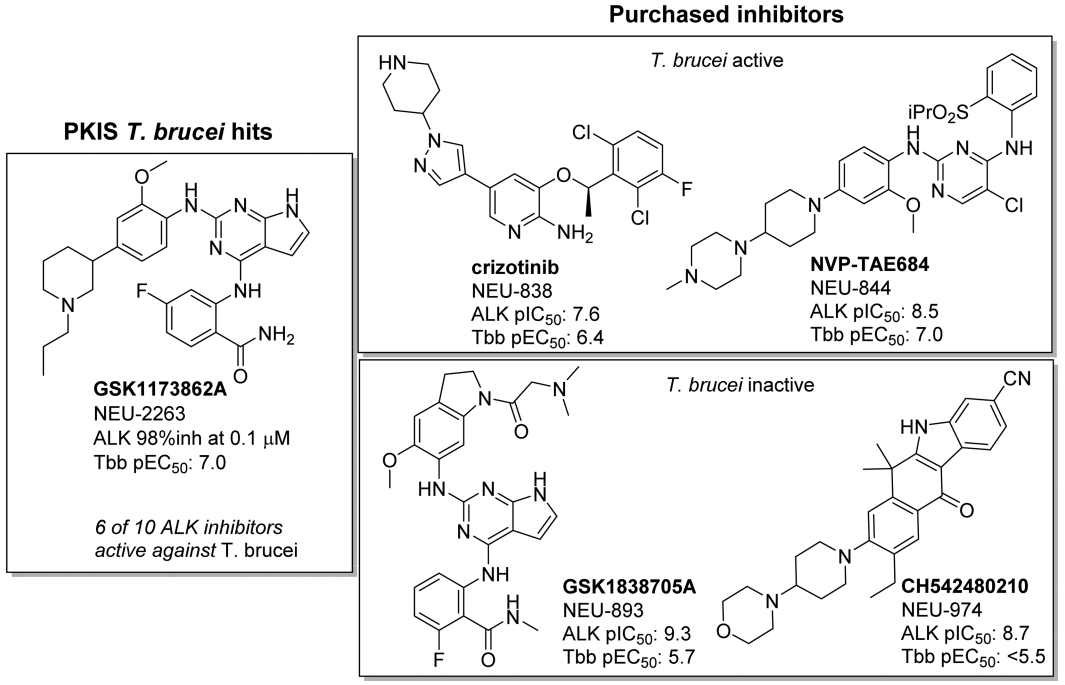

Figure 3. Progression of a representative inhibitor class from a preferred human kinase to other ALK chemotypes with *T. brucei* proliferation activity.

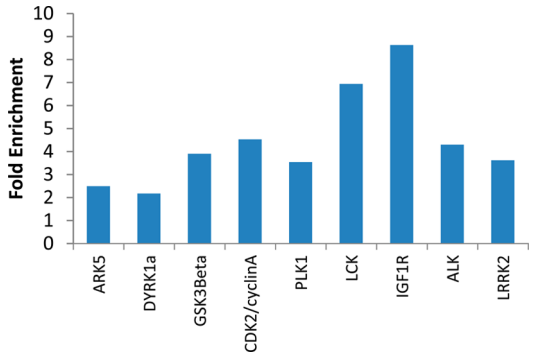

Figure 4. Fold enrichment of hit rate in *T. brucei* for actives in different preferred kinases utilizing the 80% human kinase inhibition cutoff. Data from single-concentration assays were retrieved (at least 100 compounds shared between *T. brucei* screen and kinase screen).

sourced ethically, and their research use was in accord with the terms of the informed consent.

Statistical Analyses. A 2×2 contingency table was constructed, crossing *T. brucei* "active/inactive" with human kinase "active/inactive" (see Supporting Information, Figure S1). A total number of 2016 contingency tables were generated (224 kinases × 3 kinase % inhibition cutoffs × 3 pEC_{50} cutoffs), which considered 81760 *T. brucei* activit−-human kinase activity combinations (365 × 224).

From these tables, two conditional probabilities have been calculated as reported below:

$$\text{pct1} = \frac{\text{Tb}^{+}|\text{Kinase}^{+}}{\text{Tb}^{+}|\text{Kinase}^{+} + \text{Tb}^{+}|\text{Kinase}^{-}}$$

$$\text{pct2} = \frac{\text{Tb}^{-}|\text{Kinase}^{+}}{\text{Tb}^{-}|\text{Kinase}^{+} + \text{Tb}^{-}|\text{Kinase}^{-}}$$

Finally, fold enrichment has been calculated as ratio of the two conditional probabilities:

$$\text{fold enrichment} = \frac{\text{pct1}}{\text{pct2}}$$

If the number of compounds falling in quadrant A (Tb^{+}|$Kinase^{+}$), that is, active against the selected kinase and active against *T. brucei*, is higher than the number of compounds falling in quadrant C (Tb^{-}|$Kinase^{+}$), that is, active against the selected kinase but inactive against *T. brucei*, the fold enrichment will approach values higher than 1, whereas in the opposite cases the fold enrichment for that particular human kinase will be lower than 1. We elected to utilize the *T. brucei* $pEC_{50} \geq 6$ cutoff, which provided a large enough sample to provide a statistically valid analysis, thus reducing the number of contingency tables to 672 (224 kinases × 3 kinase % inh cutoffs × 1 pEC_{50} cutoff). *p* values (chi-squared test) were calculated using R version 2.15.2 (2012-10-26).

Inhibitor Procurement. Compounds were manually selected from Selleck Chemicals on the basis of their activity against "preferred" kinases (Table S3); compounds were used as received.

Similarity Calculations. To get an idea of the structural similarity of the commercial inhibitors tested and the PKIS compounds, the Tanimoto coefficient was calculated for each inhibitor and each PKIS compound by using the RDKit cheminformatic toolkit[19] and encoding the structures by means of topological fingerprints. The results were represented as box plots using the R program.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.5b00136.

Additional figures and data tables are compiled, including the full HTS results of the PKIS compounds tested against *T. brucei* cells, and all of the screening data of the

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ACS Infect. Dis. 2016, 2, 180−186

purchased compounds against all of the parasites tested (PDF)

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Author Contributions

Contributed to experimental designs: M.P.P., M.N., E.A.. Performed statistical, computational, and cheminformatic analyses: E.A., H.X., G.C. Performed biological assays: R.G.-D., C.C.-O., M.B., P.M., J.E., N.E.R., P.J.L., S.E.L.. Wrote the manuscript: E.A., S.X., G.C., M.P.P. Contributed to data analyses: P.M., A.R., R.J.S.

Notes

All of the compound structures and assay data included in this work are also publicly available at www.collaborativedrug.com. The authors declare no competing financial interest.

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ABBREVIATIONS

PKIS, Published Kinase Inhibitor Set; NTD, neglected tropical disease; HAT, human African trypanosomiasis; GSK, GlaxoSmithKline; HTS, high-throughput screening

REFERENCES

(1) The Global Burden of Disease: 2012 Update; http://www.who.int/entity/healthinfo/global_burden_disease (accessed Jan 23, 2012).

(2) Kuepfer, I., Schmid, C., Allan, M., Edielu, A., Haary, E. P., Kakembo, A., Kibona, S., Blum, J., and Burri, C. (2012) Safety and efficacy of the 10-day melarsoprol schedule for the treatment of second stage rhodesiense sleeping sickness. *PLoS Neglected Trop. Dis. 6*, e1695.

(3) Pollastri, M. P., and Campbell, R. K. (2011) Target repurposing for neglected diseases. *Future Med. Chem. 3*, 1307−1315.

(4) Nallan, L., Bauer, K. D., Bendale, P., Rivas, K., Yokoyama, K., Horney, C. P., Pendyala, P. R., Floyd, D., Lombardo, L. J., Williams, D. K., Hamilton, A., Sebti, S., Windsor, W. T., Weber, P. C., Buckner, F. S., Chakrabarti, D., Gelb, M. H., and Van Voorhis, W. C. (2005) Protein farnesyltransferase inhibitors exhibit potent antimalarial activity. *J. Med. Chem. 48*, 3704−3713.

(5) Diaz-Gonzalez, R., Kuhlmann, F. M., Galan-Rodriguez, C., Madeira da Silva, L., Saldivia, M., Karver, C. E., Rodriguez, A., Beverley, S. M., Navarro, M., and Pollastri, M. P. (2011) The susceptibility of trypanosomatid pathogens to PI3/mTOR kinase inhibitors affords a new opportunity for drug repurposing. *PLoS Neglected Trop. Dis. 5*, e1297.

(6) Ochiana, S. O., Pandarinath, V., Wang, Z., Kapoor, R., Ondrechen, M. J., Ruben, L., and Pollastri, M. P. (2013) The human Aurora kinase inhibitor danusertib is a lead compound for anti-trypanosomal drug discovery via target repurposing. *Eur. J. Med. Chem. 62*, 777−784.

(7) Patel, G., Karver, C. E., Behera, R., Guyett, P. J., Sullenberger, C., Edwards, P., Roncal, N. E., Mensa-Wilmot, K., and Pollastri, M. P. (2013) Kinase scaffold repurposing for neglected disease drug discovery: discovery of an efficacious, lapatinib-derived lead compound for trypanosomiasis. *J. Med. Chem. 56*, 3820−3832.

(8) Patel, G., Roncal, N. E., Lee, P. J., Leed, S. E., Erath, J., Rodriguez, A., Sciotti, R. J., and Pollastri, M. P. (2014) Repurposing human Aurora kinase inhibitors as leads for anti-protozoan drug discovery. *MedChemComm 5*, 655−658.

(9) Seixas, J. D., Luengo-Arratta, S. A., Diaz, R., Saldivia, M., Rojas-Barros, D. I., Manzano, P., Gonzalez, S., Berlanga, M., Smith, T. K., Navarro, M., and Pollastri, M. P. (2014) Establishment of a structure-activity relationship of the NVP-BEZ235 chemotype as a lead for African sleeping sickness. *J. Med. Chem. 57*, 4834−4848.

(10) Diaz, R., Luengo-Arratta, S. A., Seixas, J. D., Amata, E., Devine, W., Cordon-Obras, C., Rojas-Barros, D. I., Jimenez, E., Ortega, F., Crouch, S., Colmenarejo, G., Fiandor, J. M., Martin, J. J., Berlanga, M., Gonzalez, S., Manzano, P., Navarro, M., and Pollastri, M. P. (2014) Identification and characterization of hundreds of potent and selective inhibitors of *Trypanosoma brucei* growth from a kinase-targeted library screening campaign. *PLoS Neglected Trop. Dis. 8*, e3253.

(11) Dranchak, P., MacArthur, R., Guha, R., Zuercher, W. J., Drewry, D. H., Auld, D. S., and Inglese, J. (2013) Profile of the GSK published protein kinase inhibitor set across ATP-dependent and-independent luciferases: implications for reporter-gene assays. *PLoS One 8*, e57888.

(12) Drewry, D. H., Willson, T. M., and Zuercher, W. J. (2014) Seeding collaborations to advance kinase science with the GSK Published Kinase Inhibitor Set (PKIS). *Curr. Top. Med. Chem. 14*, 340−342.

(13) Ochiana, S. O., Pandarinath, V., Wang, Z., Kapoor, R., Ondrechen, M. J., Ruben, L., and Pollastri, M. P. (2013) The human Aurora kinase inhibitor danusertib is a lead compound for anti-trypanosomal drug discovery via target repurposing. *Eur. J. Med. Chem. 62*, 777−784.

(14) Urbaniak, M. D., Mathieson, T., Bantscheff, M., Eberhard, D., Grimaldi, R., Miranda-Saavedra, D., Wyatt, P., Ferguson, M. A. J., Frearson, J., and Drewes, G. (2012) Chemical proteomic analysis reveals the drugability of the kinome of *Trypanosoma brucei*. *ACS Chem. Biol. 7*, 1858−1865.

(15) Parsons, M., Worthey, E. A., Ward, P. N., and Mottram, J. C. (2005) Comparative analysis of the kinomes of three pathogenic trypanosomatids: *Leishmania major*, *Trypanosoma brucei* and *Trypanosoma cruzi*. *BMC Genomics 6*, 127.

(16) Behera, R., Thomas, S. M., and Mensa-Wilmot, K. (2014) New chemical scaffolds for human african trypanosomiasis lead discovery from a screen of tyrosine kinase inhibitor drugs. *Antimicrob. Agents Chemother. 58*, 2202−2210.

(17) Patefield, W. M. (1981) Algorithm AS159. An efficient method of generating r × c tables with given row and column totals. *Appl. Stat. 30*, 91−97.

(18) Agresti, A. (2007) *An Introduction to Categorical Data Analysis*, 2nd ed., Wiley, New York.

(19) RDKit; http://www.rdkit.org.

DOI: 10.1021/acsinfecdis.5b00136
ACS Infect. Dis. 2016, 2, 180−186