Discovery of Ligand-Efficient Scaffolds for the Design of Novel \textit{Trichomonas vaginalis} Uridine Nucleoside Ribohydrolase Inhibitors Using Fragment Screening

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Supporting Information

ABSTRACT: Trichomoniasis is caused by the parasitic protozoan \textit{Trichomonas vaginalis}. The increasing prevalence of strains resistant to the current 5-nitroimidazole treatments creates the need for novel therapies. \textit{T. vaginalis} cannot synthesize purine and pyrimidine rings and requires salvage pathway enzymes to obtain them from host nucleosides. The uridine nucleoside ribohydrolase was screened using an \textsuperscript{19}F NMR-based activity assay against a 2000-compound fragment diversity library. Several series of inhibitors were identified including scaffolds based on acetamides, cyclic ureas or ureas, pyridines, and pyrrolidines. A number of potent singleton compounds were identified, as well. Eighteen compounds with IC\textsubscript{50} values of 20 μM or lower were identified, including some with ligand efficiency values of 0.5 or greater. Detergent and jump-dilution counter screens validated all scaffold classes as target-specific, reversible inhibitors. Identified scaffolds differ substantially from 5-nitroimidazoles. Medicinal chemistry using the structure–activity relationship emerging from the fragment hits is being pursued to discover nanomolar inhibitors.

INTRODUCTION

Trichomoniasis is the most common sexually transmitted nonviral infection worldwide.\textsuperscript{1} The World Health Organization estimated that there were 276.4 million cases in 2008 with 90% of these cases occurring in resource-limited areas.\textsuperscript{1} In the United States, an estimated 3.7 million cases were reported by the Centers for Disease Control and Prevention.\textsuperscript{2} Once infected, a person is more likely to become infected with chlamydia, gonorrhea, herpes simplex viruses type-1 and type-2, HIV, syphilis, and other sexually transmitted diseases.\textsuperscript{3–4} Infections also increase the risk of developing bacterial vaginosis, candidiasis, pelvic inflammatory disease, and cervical and prostate cancer; pregnant women infected with trichomoniasis have an increased risk for low birth weight and preterm delivery.\textsuperscript{5,6} Trichomonas is caused by the parasite \textit{Trichomonas vaginalis}, a flagellated protozoan that is pyriform in shape. The majority of the time, \textit{T. vaginalis} inhabits the squamous epithelium of the genital tract, performing fermentation using carbohydrates under both aerobic and anaerobic conditions.\textsuperscript{5}

The current treatments for \textit{T. vaginalis} infection are 5-nitroimidazoles.\textsuperscript{5,6} This class of compounds, including metronidazole and tinidazole, is activated in the parasite’s hydrogenosomes; the nitro group is reduced by pyruvate−ferredoxin oxidoreductase creating toxic nitro radical anions, which damage thymine and adenine residues in the parasite’s DNA, causing the DNA to be cleaved and subsequent parasitic death.\textsuperscript{5,6} Infections resulting from 5-nitroimidazole-resistant strains of \textit{T. vaginalis}, however, are becoming more widespread, accounting for 5−17% of infections depending on the country.\textsuperscript{5,6} New antichromosomal agents with a mechanism of action distinct from existing drugs would provide a second line of therapy and would improve outcomes for the increasing number of patients with drug-resistant \textit{T. vaginalis} infections.

Potential antichromosomal drug targets include purine and pyrimidine salvage pathway enzymes. \textit{T. vaginalis} lacks de novo biosynthetic pathways for purine and pyrimidine rings and relies on salvage pathway enzymes to metabolize nucleosides harvested from host cells.\textsuperscript{8−10} The first step in these salvage pathways is the hydrolysis of nucleosides into their nucleobase and ribose components. The responsible enzymes belong to a superfAMILY of structurally related calcium-containing nucleoside ribohydrolases (NHs).\textsuperscript{11} Previous studies have shown that all NHs have an active site highly specific to ribose, while...
specificity for the nucleobases is highly variable.\textsuperscript{11} Despite the variability in substrate specificity of NHs, all enzymes of this class contain a Ca\textsuperscript{2+} cation deep within their active site coordinated by several conserved aspartic acid residues. The ribose portion of the nucleoside coordinates the Ca\textsuperscript{2+} cation via its 2′- and 3′-hydroxyl groups positioning the glycosidic bond for hydrolysis. A water molecule is also coordinated by the Ca\textsuperscript{2+} cation activating it for base-catalyzed hydrolysis of the N-glycosidic bond.\textsuperscript{11}

While the ribose pockets of NH structures are highly conserved, the nucleobase pocket is more variable. The \textit{T. vaginalis} genome\textsuperscript{12} contains three confirmed NHs that we have cloned and characterized. Two are specific for purines, adenosine/guanosine nucleoside hydrolase (AGNH)\textsuperscript{13} and guanosine/adenosine/cytidine nucleoside hydrolase (GACNH),\textsuperscript{14} while the third is specific for pyrimidines, uridine nucleoside hydrolase (UNH).\textsuperscript{15} Expressed sequence tags have been reported for all three \textit{NHs}.\textsuperscript{16} The transcriptome of \textit{NHs} under anaerobic conditions has been compared to that after exposure to oxygen and to vaginal epithelial cells\textsuperscript{17} and has also been studied in response to glucose restriction.\textsuperscript{18} Interestingly, transcripts for UNH were found to be up to 50-fold greater in number than those for either AGNH or GACNH depending on growth conditions. This might indicate the unique role played by this pyrimidine nucleoside ribohydrolase. Pyrimidine metabolism has been extensively studied in the related parasite \textit{Trypanosoma brucei}, which, in contrast to \textit{T. vaginalis}, is capable of both de novo biosynthesis and salvage of pyrimidines.\textsuperscript{19} However, \textit{T. brucei} genetically modified to lack de novo pyrimidine biosynthesis capability was found to be completely dependent on salvage pathways, with the absence of pyrimidines in growth media rapidly lethal.\textsuperscript{20} The addition of uracil returned growth rates to normal, while the addition of uridine only partially restored growth rates. This provides strong evidence that inhibiting the pyrimidine salvage pathway in \textit{T. vaginalis} will be lethal to the parasite since this pathway represents its sole pyrimidine source.

We previously determined that UNH is a druggable target by developing an \textsuperscript{19}F NMR-based activity assay and then using it to screen the National Institutes of Health (NIH) Clinical Compound Collection for inhibitors.\textsuperscript{15} Although the compounds in this collection have relatively large molar masses and lack chemical diversity, several benzimidazole-containing proton–pump inhibitors were identified as low micromolar inhibitors including omeprazole shown in Figure 1. Omeprazole has an \textit{IC}_{50} value of 2.3 \textmu M, but its relatively large molar mass of 345 g/mol combined with only modest ligand efficiency (LE)\textsuperscript{21,22} of 0.36 (heavy atom count of 24) makes it a poor starting point for drug design.\textsuperscript{23} A small hit deconstruction effort identified the fragment 2-methylthiobenzimidazole shown in Figure 1 that has a molar mass of 164 g/mol and a much higher LE of 0.53 (heavy atom count of 11). These metrics suggest that 2-methylthiobenzimidazole could potentially be developed into a nanomolar inhibitor with a final molar mass less than 500 Da.\textsuperscript{23,24} The relatively easy hit deconstruction of a modestly potent compound identified from a sampling of limited chemical diversity suggested that screening a large and diverse fragment library might identify multiple structure classes with better prospects for medicinal chemistry efforts.

\section*{RESULTS AND DISCUSSION}

The \textsuperscript{19}F NMR-based activity assay monitors the conversion of 5-fluorouridine to 5-fluorouracil and ribose.\textsuperscript{15} While the reaction could also be monitored using \textsuperscript{1}H NMR of uridine/uracil, \textsuperscript{19}F NMR is advantageous because of the lack of possible overlaps with signals from the fragments themselves and its comparable sensitivity to \textsuperscript{1}H NMR.\textsuperscript{25} Further, since the same two substrate and product \textsuperscript{19}F NMR signals are observed in every reaction, the effects of relaxation and chemical shift anisotropy that can complicate ligand-based \textsuperscript{19}F NMR screening methods are not a concern here.\textsuperscript{26} At the 333 \mu M fragment concentrations screened, a competitive fragment inhibitor would need to have a \textit{K}_i of only 77 \mu M to result in 50% inhibition.

Mixtures of six fragments were initially screened, with mixtures that exhibited at least 75% inhibition subsequently deconvoluted to determine the individual inhibitory fragments. Figure 2 shows typical spectra for six mixtures along with the 0 and 30 min control spectra. Only the substrate signal at -165.8 ppm is observed in the 0 min control spectrum, while both the substrate signal and a new product signal at -169.2 ppm are observed in the 30 min control spectrum. The product peak is also present in all mixture spectra except that for mixture 5 indicating that at least one compound in mixture 5 is an inhibitor of UNH. Figure 3 shows the spectra for the deconvolution of mixture 5 using its six individual components. The product peak is present in all compounds tested with the exception of fragment G7. The absence of the product peak at -169.2 ppm indicates that fragment G7 fully inhibits UNH at 333 \mu M. The observation of residual substrate signals for fragments C8 and D8 indicates that these fragments are also inhibitors but much weaker. Fragments demonstrating

\begin{figure}[h]
\begin{center}
\includegraphics[width=0.5\textwidth]{figure1.png}
\end{center}
\caption{Structures, potencies, and ligand efficiencies of omeprazole and its fragment 2-methylthiobenzimidazole.}
\end{figure}
75% or greater inhibition in the deconvolution assays were assayed in five-point serial dilutions down to 1.3 μM. Fragment IC₅₀ values or percent inhibition at 333 μM were then determined. A total of 33 fragments selected to represent the various chemical classes of inhibitors were obtained as solid samples to confirm activity. These compounds were dissolved in dimethyl sulfoxide (DMSO) and retested from 1.33 mM to 0.33 μM, as shown in Figure 4 for fragment G7. The IC₅₀ value for fragment G7 (subsequently referred to as fragment 7) calculated from this data is 45 μM.

A total of 97 fragments exhibited inhibition against UNH (4.9% hit rate). Several series of inhibitors with emerging structure–activity relationship (SAR) were identified including scaffolds based on acetamides, cyclic ureas or ureas, pyridines, and pyrrolidines. A number of potent singleton compounds were identified, as well. A singleton was defined as having no other closely related fragments in the screen, based on substructure searching of the core scaffold. Among the active fragments were 18 compounds with IC₅₀ values of 20 μM or lower, including some with ligand efficiency values of 0.5 or greater. The structures, IC₅₀ values, and LE values for nine fragments representative of the most common scaffolds are shown in Table 1. A total of 55 structural analogs of the most potent fragments were also obtained and tested. The emerging SAR from these fragments and the original screening hits are discussed below.

Interestingly, several of the fragment classes contain moieties that are components of active compounds identified in our previous screen of the NIH Clinical Compound Collection. For instance, the 2,3-substituted pyridine ring of fragments 4 and 5 is one component of the prazole class of compounds represented by omeprazole in Figure 1. The phenylpyridine component of fragment 7 is also very nearly the core 4-phenyl-1,4-dihydropyridine scaffold of the dipine compounds, such as nifedipine and nicardipine, which were the largest class of hits in the previous screen. Thus, the SAR from the previous NIH Clinical Compound Collection can be integrated in some circumstances in the context of the SAR from this emerging work to advance selected hit series.

All compound structural classes were validated as reversible, target-specific inhibitors based on four criteria. First, the fragment library was designed to exclude PAINS chemotypes, and this was verified for the fragments shown in Table 1 using ZINC. Further, the fragments shown in Table 1 were analyzed by the program Badapple, an algorithm that detects likely patterns of promiscuity in molecular scaffolds. High scores were indicated only for fragments containing biphenyl, phenylpyrrolidine, and pyridine chemotypes. Second, the lack of reporter enzymes in the NMR-based activity assays eliminates the possibility of false positives acting by this interference mechanism. Third, detergent counter screens were carried out to reduce the incidence of false positives arising.

### Table 1. Structures, IC₅₀ Values, and LE Values of the Most Common Fragment Scaffolds

| Fragment | Structure | IC₅₀ (μM)ᵃ | LE  |
|----------|-----------|------------|-----|
| 1        | ![Fragment 1](image) | 32         | 0.44|
| 2        | ![Fragment 2](image) | 3.6        | 0.44|
| 3        | ![Fragment 3](image) | 64         | 0.44|
| 4        | ![Fragment 4](image) | 92         | 0.40|
| 5        | ![Fragment 5](image) | 14         | 0.50|
| 6        | ![Fragment 6](image) | 86         | 0.38|
| 7        | ![Fragment 7](image) | 45         | 0.40|
| 8        | ![Fragment 8](image) | 150        | 0.43|
| 9        | ![Fragment 9](image) | 45         | 0.49|

ᵃValues are the average of n = 2.
from colloidal aggregates that can mimic inhibition by blocking the enzyme’s active site. The Shoichet protocol was used to test for aggregation-based inhibition, where the nonionic detergent Triton X-100 will prevent aggregates from interacting with the enzyme nonspecifically. If activity diminishes markedly with detergent, the compound is most likely an aggregator. Figure 5 demonstrates the effect of detergent on the inhibition observed for fragment 7. Both control reactions have approximately 50% conversion of substrate to product, while reactions with 100 μM fragment 7 in the absence and presence of 0.01% Triton X-100 detergent show close to complete inhibition. Lack of significant change in potency with or without detergent indicates that the inhibition observed for fragment 7 is likely not aggregation-based. Similar results were obtained for all other fragments tested, indicating that all classes are target-specific inhibitors. In these experiments, 19F NMR is actually disadvantageous compared to 1H NMR. When using the latter, resonances from the fragments themselves are often simultaneously indicative of well-behaved, soluble compounds. Fourth, jump-dilution assays were carried out to confirm that the fragment hits are noncovalent, reversible inhibitors. Jump-dilution assays include a parallel incubation of the enzyme and compound at 200 μM before initiating normal reaction assays at 200 and 20 μM (10-fold dilution). Fragment 7 completely inhibited UNH at 200 μM, as shown in Figure 6. Full inhibition is expected since the fragment concentration is 4-fold higher than its IC50 value of 45 μM. However, upon rapid dilution to 20 μM before initiating the reaction, UNH inhibition dropped to 54%, as shown in Figure 6. Loss of activity indicates dissociation of the compound from the active site. Similar results were obtained for all other fragments tested, indicating that all classes are noncovalent, reversible inhibitors. Fragment SAR and dose–response curve shapes also suggest that the identified fragments are suitable for follow-up studies.

The impetus to screen a fragment diversity library came from the observation that fragments with high LE values could be identified from larger-molecular-weight inhibitors, as shown in Figure 1. Screening a large set of diverse fragments might then lead to the identification of one or more scaffolds more optimal for medicinal chemistry elaboration. Identification of scaffold classes with more than five representatives indicates the success of the fragment approach and provides excellent starting points for ongoing work. Further, the scaffold classes identified are markedly different from those identified in our previous fragment screen of the same library against the purine-specific AGNH enzyme. Of the 60 fragments with IC50 values <100 μM for UNH, only nine also had IC50 values <100 μM for AGNH. This suggests that while the ribose binding pockets of the AGNH and UNH active sites are likely very similar, the nucleobase binding pockets possess markedly different molecular complementarities.

Several scaffold classes contain fragments with LE values greater than 0.5, indicating that the majority of the atoms make favorable interactions in the active site. It is important to start out with high LE values since during the optimization process, the efficiency will only remain the same or decrease as the size of the molecule is increased. For instance, the molar masses and LE values for all of the fragments in Table 1 with the exception of fragment 6 suggest that they can each be developed into nanomolar inhibitors with final molar masses less than 500 Da provided that LE remains constant as molar mass increases. Thus, the fragment screening output provides at least four chemical scaffolds that are attractive starting points for a chemical optimization program. Some fragments and fragment classes also appear to have overlapping structural features that may suggest fragment merging strategies, as well.

The 3-hydroxypropylidine fragment 9 is compelling for its combination of potency, LE, and emerging SAR. Figure 7 compares the structure of fragment 9 with that of uridine. Interestingly, the inhibitor has almost one-third greater LE than the enzyme’s natural substrate. The lower LE for the substrate results from some of the binding energy being used to lower the activation energy for the reaction, thus reducing binding affinity. Inhibitors (nonsubstrates) do not have this limitation and thus can have higher LE values. There are, at present, no reported structures of pyrimidine-specific nucleoside hydrolases with a bound heterocyclic nucleobase in the
active site. However, modeling studies on the pyrimidine-specific enzymes from *Escherichia coli* and *Sulfolobus solfataricus* indicate that both contain hydrophilic residues lining the active site that could potentially hydrogen-bond with the polar regions of fragment 9 and the substrate. In addition, both fragment 9 and uridine have 6-membered aromatic/heteroaromatic rings that have a hydrophobic face that may make similar interactions in the active site. Both structures also contain 5-membered, saturated rings with attached hydroxyl groups. The pyrrolidine moiety of fragment 9 likely interacts with the ribose pocket of the active site. As previously discussed, nucleoside hydrolases have a highly conserved Ca\(^{2+}\) cation within their active sites. Fragment 9 has a hydroxyl group in a similar position as uridine. It is highly probable that this hydroxyl group is interacting with the Ca\(^{2+}\) cation in the same manner as the 2′ hydroxyl group in uridine. Modeling and X-ray crystallography studies to validate these interactions and guide inhibitor design are in progress.

A total of six 3-hydroxypyrrolidines were identified as inhibitors including fragments 8 and 9 from the original fragment library and fragments 10−13 shown in Table 2 from the fragment hit structural analogs tested. Replacement of the methylamino group in fragment 9 with a nitrile group in fragment 10 improved potency, with fragment 10 having an IC\(_{50}\) value of 13 μM. By contrast, the addition of two meta methyl groups as in fragment 11, a para ethyl group as in fragment 8, and an ethoxy group as in fragment 12 resulted in a steady decrease in potency compared to that in fragment 9. This suggests either a steric hindrance or a limit to the hydrophobic character in this region of the active site and that the nonpolar edge of the uracil-like ring is a poor vector for picking up new interactions. Further, the pyridine ring of fragment 13 had only barely detectable activity suggesting that the ring nitrogen is in the wrong position to pick up similar interactions in the active site that are responsible for substrate specificity. Adding hydrogen-bonding groups that mimic those of the uracil ring may improve activity.

### CONCLUSIONS

Fragment hits identified here provide ideal starting points to synthesize the tool compounds required to demonstrate that UNH inhibition is correlated with antitrichomonal activity. This will require improvements in UNH potency by several orders of magnitude, down into the 10 nM range. In addition to having small molecular weight and favorable aqueous solubility, the diverse compounds in the fragment library were selected for their potential to be elaborated on using medicinal chemistry protocols. Thus, the scaffolds identified with high ligand efficiencies can be chemically expanded using known synthetic organic chemistry approaches. This process will enable the development of larger compounds with improved UNH activity that meet the criteria for in vitro testing. Inhibitors active against *T. vaginalis* may also be broadly applicable to other neglected parasites that require nucleoside hydrolase enzymes for their survival such as *Leishmania donovani*.

### EXPERIMENTAL SECTION

NMR data sets were acquired on a Bruker AvanceIII 500 MHz spectrometer using a BBFO probe. \(^{19}F\{1H\} \) NMR spectra were acquired using inverse-gated decoupling with WALTZ-16. Spectra were the average of 256 scans and included acquisition and relaxation delay times of 0.872 s and 4.0 s, respectively. \(^{19}F\) chemical shifts were referenced to external 50 μM trifluoroethanol at −76.7 ppm. The physical properties of the 1963 fragments screened, a diversity-based subset of the AstraZenea fragment library, have been described previously.

Sequentially, 3 μL of 10 mM 5-fluourouridine and 2 μL of each fragment to be tested were added to microfuge tubes. Reactions were then initiated using a stock solution consisting of 50 mM potassium phosphate and 0.3 M KCl at pH 6.5, 10% \(^2\)H\(_2\)O, and 80 nM UNH to give a final volume of 600 μL. Reactions were quenched after 30 min with 10 μL of 1.5 M HCl. The highest DMSO concentrations used were 2%, which did not measurably affect enzyme activity. In all cases, control reactions were also run by quenching at 0 and 30 min in the presence of the same DMSO concentration but the absence of fragments. Serial dilution assays were carried out in duplicate and analyzed as previously described, maintaining a constant DMSO concentration for each dilution.

Jump-dilution and detergent counter screens were carried out as previously described. The IC\(_{50}\) values of the fragments used in these experiments were well suited to the 200 and 20 μM fragment concentrations used in the jump-dilution assays, as well as the 100 and 50 μM fragment concentrations used in the detergent assays.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02472.

Jump-dilution and detergent counter screen assay data for eight compounds; two panels of NMR spectra are...
shown for each type of counter screen (Figures S1–S9); counter screen assay data summary (Table S1) (PDF)

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Notes
The authors declare no competing financial interest.

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REFERENCES

(1) Hirt, R. P.; Sherard, J. Trichomonas vaginalis origins, molecular pathobiology and clinical considerations. Curr. Opin. Infect. Dis. 2015, 28, 72–79.
(2) Bouchemal, K.; Bories, C.; Loiseau, P. M. Strategies for prevention and treatment of Trichomonas vaginalis infections. Clin. Microbiol. Rev. 2017, 30, 811–825.
(3) Kissinger, P. Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues. BMC Infect. Dis. 2015, 15, 307–314.
(4) Leitsch, D. Recent advances in the treatment of Trichomonas vaginalis. F1000Research 2016, 5, No. 162.
(5) Secor, W. E. Trichomonas vaginalis: treatment questions and challenges. Expert Rev. Anti-Infect. Ther. 2012, 10, 107–109.
(6) Cudmore, S. L.; Delgaty, K. L.; Hayward-McClelland, S. F.; Petrin, D. P.; Garber, G. E. Treatment of infections caused by metronidazole-resistant Trichomonas vaginalis. Clin. Microbiol. Rev. 2004, 17, 783–793.
(7) Conrad, M. D.; Bradic, M.; Warring, S. D.; Gorman, A. W.; Carlton, J. M. Getting trichy: Tools and approaches to interrogating Trichomonas vaginalis in a post-genome world. Trends Parasitol. 2013, 29, 17–25.
(8) Heyworth, P. G.; Gutteridge, W. E.; Ginger, C. D. Purine metabolism in Trichomonas vaginalis. FEBS Lett. 1982, 141, 106–110.
(9) Wang, C. C.; Cheng, H-W. Salvage of pyrimidine nucleosides by Trypanosoma brucei. Mol. Biochem. Parasitol. 1984, 10, 171–184.
(10) Heyworth, P. G.; Gutteridge, W. E.; Ginger, C. D. Pyrimidine metabolism in Trichomonas vaginalis. FEBS Lett. 1984, 176, 55–60.
(11) Versées, S.; Weystaet, J. Catalysis by nucleoside hydrolases. Curr. Opin. Struct. Biol. 2003, 13, 731–738.
(12) Carlton, J. M.; Hirt, R. P.; Silva, J. C.; Delcher, A. L.; Schatz, M.; Zhao, Q.; Wortman, J. R.; Bidwell, S. L.; Alsmark, U. C. M.; Besteiro, S.; Sichertz-Ponten, T.; Noel, C. J.; Dacks, J. B.; Foster, P. G.; Simillion, C.; Van de Peer, Y.; Miranda-Saavedra, D.; Barton, G. J.; Westrop, G. D.; Muller, S.; Desii, D.; Fiori, P. L.; Ren, Q.; Paulsen, L.; Zhang, H.; Bastida-Corcuera, F. D.; Simoes-Barbosa, A.; Brown, M. T.; Hayes, R. D.; Mukherjee, M.; Okumura, C. Y.; Schneider, R.; Smith, A. J.; Vanacova, S.; Villalvazo, M.; Haas, B. J.; Perta, M.; Feldblum, T. V.; Utterback, T. R.; Shu, C.-L.; Osogawa, K.; de Jong, P. J.; Hrdy, I.; Horvathova, L.; Zubicova, Z.; Dolezal, P.; Malik, S.-B.; Logsdon, J. M., Jr.; Henze, K.; Gupta, A.; Wang, C. C.; Dunne, R. L.; Upcroft, J. A.; Upcroft, P.; White, O.; Salberg, S. L.; Tang, P.; Chiu, C.-H.; Lee, Y.-S.; Embley, T. M.; Coombs, G. H.; Mottram, J. C.; Tachezy, J.; Fraser-Liggett, C. M.; Johnson, P. J. Draft genome sequence of the sexually transmitted pathogen Trichomonas vaginalis. Science 2007, 315, 207–212.
(13) Beck, S.; Muellers, S. N.; Benzie, A. L.; Parkin, D. W.; Stockman, B. J. Adenosine/guanosine preferring nucleoside ribohydrolase is a distinct, druggable antitrichomonal target. Bioorg. Med. Chem. Lett. 2015, 25, 5036–5039.
(14) Alam, R.; Barbarovich, A. T.; Caravan, W.; Imsland, M.; Barskaya, A.; Parkin, D. W.; Stockman, B. J. Drugability of the guanosine/adenosine/cytidine nucleoside hydrolase from Trichomonas vaginalis. Chem. Biol. Drug Des. 2018, 92, 1736–1742.
(15) Shea, T. A.; Burburan, P. J.; Matubia, V. N.; Ramcharan, S. S.; Rosario, L. Jr.; Parkin, D. W.; Stockman, B. J. Identification of proton-pump inhibitor drugs that inhibit Trichomonas vaginalis uridine nucleoside ribohydrolase. Bioorg. Med. Chem. Lett. 2014, 24, 1080–1084.
(16) Aurrecoechea, C.; Brestelli, J.; Brunk, B. P.; Carlton, J. M.; Dommer, J.; Fischer, S.; Gajria, B.; Gao, X.; Gingle, A.; Grant, G.; Harb, O. S.; Heiges, M.; Innamorato, F.; Iodice, J.; Kissinger, J. C.; Kraemer, E.; Li, W.; Miller, J. A.; Morrison, H. G.; Nayak, V.; Pennington, C.; Pinney, D. F.; Roos, D. S.; Ross, C.; Stoeckert, C. J., Jr.; Sullivan, S.; Treatman, C.; Wang, H. GiardiaDB and TrichDB: integrated genomic resources for the eukaryotic protist pathogens Giardia lamblia and Trichomonas vaginalis. Nucleic Acids Res. 2009, 37, DS26–DS30.
(17) Gould, S. B.; Woehle, C.; Kusdian, G.; Landan, G.; Tachezy, J.; Zimorski, V.; Martin, W. F. Deep sequencing of Trichomonas vaginalis during the early infection of vaginal epithelial cells and amoeboid transition. Int. J. Parasitol. 2013, 43, 707–719.
(18) Huang, K.-Y.; Chen, Y.-Y. M.; Fang, Y.-K.; Cheng, W.-H.; Cheng, C.-C.; Chen, Y.-C.; Wu, T. E.; Ku, F.-M.; Chen, S.-C.; Lin, R.; Tang, P. Adaptive responses to glucose restriction enhance cell survival, antioxidant capability, and autophagy of the protozoan parasite Trichomonas vaginalis. Biochim. Biophys. Acta, Gen. Subj. 2014, 1840, 53–64.
(19) Hammond, D. J.; Gutteridge, W. E. Purine and pyrimidine metabolism in the trypanosomatidae. Mol. Biochem. Parasitol. 1984, 13, 243–261.
(20) Ali, J. A. M.; Tagoe, D. N. A.; Munday, J. C.; Donachie, A.; Morrison, L. J.; de Koning, H. P. Pyrimidine biosynthesis is not an essential function for Trypanosoma brucei bloodstream forms. PLoS One 2013, 8, e68034.
(21) Hopkins, A. L.; Groom, C. R.; Alex, A. Lindag efficiency: a useful metric for lead selection. Drug Discov. Today 2004, 9, 430–431.
(22) Abad-Zapatero, C. Lindag efficiency indices for effective drug discovery. Expert Opin. Drug Discovery 2007, 2, 469–488.
(23) Hajduk, P. J. Fragment-based drug design: How big is too big? J. Med. Chem. 2006, 49, 6972–6976.
(24) Valdez-Padilla, D.; Rodriguez-Morales, S.; Hernández-Campos, A.; Hernández-Luis, F.; Yépez-Mulia, L.; Tapia-Contreras, A.; Castillo, R. Synthesis and antiprotozoal activity of novel 1-methylbenzimidazole derivatives. Bioorg. Med. Chem. 2009, 17, 1724–1730.
(25) Dalvit, C.; Ardini, E.; Flocco, M.; Fogliato, G. P.; Mongelli, N.; Veronesi, M. A general NMR method for rapid, efficient, and reliable biochemical screening. J. Am. Chem. Soc. 2003, 125, 14620–14625.
(26) Dalvit, C. Lindag- and substrate-based 19F NMR screening: Principles and applications to drug discovery. Prog. Nucl. Magn. Reson. Spectrosc. 2007, 51, 243–271.
(27) Aldrich, C.; Bertozzi, C.; Georg, G. I.; Kiesling, L.; Lindsey, C.; Liotta, D.; Merz, K. M.; Schepert, A.; Wang, S. The ecstacy and agony of assay interference compounds. ACS Cent. Sci. 2017, 3, 143–147.
(28) Baell, J. B.; Holloway, G. A. New substructure filters for removal of pan assay interference compounds (PAINS) from...
screening libraries and for their exclusion in bioassays. *J. Med. Chem.* 2010, 53, 2719–2740.

(29) Sterling, T.; Irwin, J. J. ZINC 15 − ligand discovery for everyone. *J. Chem. Inf. Model.* 2015, 55, 2324−2337.

(30) Yang, J. J.; Ursu, O.; Lipinski, C. A.; Sklar, L. A.; Oprea, T. I.; Bologa, C. G. Badapple: promiscuity patterns from noisy evidence. *J. Cheminform.* 2016, 8, 29−42.

(31) Feng, B. Y.; Shoichet, B. K. A detergent-based assay for the detection of promiscuous inhibitors. *Nat. Protoc.* 2006, 1, 550−553.

(32) Stockman, B. J.; Kaur, A.; Persaud, J. K.; Mahmood, M.; Thuilot, S. F.; Emilcar, M. B.; Canestrari, M.; Gonzalez, J. A.; Auletta, S.; Sapojnikov, V.; Caravan, W.; Muellers, S. N. NMR-based activity assays for determining compound inhibition, IC_{50} values, artifactual activity, and whole cell activity of nucleoside ribohydrolases. *J. Visualized Exp.* 2019, 148, No. e59928.

(33) Muellers, S. N.; Gonzalez, J. A.; Kaur, A.; Sapojnikov, V.; Benzie, A. L.; Brown, D. G.; Parkin, D. W.; Stockman, B. J. Ligand-efficient inhibitors of *Trichomonas vaginalis* adenosine/guanosine preferring nucleoside ribohydrolase. *ACS Infect. Dis.* 2019, 5, 345−352.

(34) Copeland, R. A.; Basavapathruni, A.; Moyer, M.; Scott, M. P. Impact of enzyme concentration and residence time on apparent activity recovery in jump dilution analysis. *Anal. Biochem.* 2011, 416, 206−210.

(35) Iovane, E.; Giabbai, B.; Muzzolini, L.; Matafora, V.; Fornili, A.; Minici, C.; Giannese, F.; Degano, M. Structural basis for substrate specificity in group I nucleoside hydrolases. *Biochemistry* 2008, 47, 4418−4426.

(36) Minici, C.; Cacciapuoti, G.; De Leo, E.; Porcelli, M.; Degano, M. New determinants in the catalytic mechanism of nucleoside hydrolases from the structures of two isozymes from *Sulfolobus solfataricus*. *Biochemistry* 2012, 51, 4590−4599.

(37) Nirma, C.; Rangel, G. T.; Alves, M. A.; Casanova, L. M.; Moreira, M. M.; Rodriguesa, L. M.; Hamerski, L.; Tinoco, L. W. New *Leishmania donovani* nucleoside hydrolase inhibitors from Brazilian flora. *RSC Adv.* 2019, 9, 18663−18669.

(38) Shaka, A. J.; Keeler, J.; Frenkiel, T.; Freeman, R. An improved sequence for broadband decoupling: WALTZ-16. *J. Magn. Reson.* 1969, 52, 335−338.