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Identification of proton-pump inhibitor drugs that inhibit *Trichomonas vaginalis* uridine nucleoside ribohydrolase

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ABSTRACT

Trichomonas vaginalis continues to be a major health problem with drug-resistant strains increasing in prevalence. Novel antitrichomonal agents that are mechanistically distinct from current therapies are needed. The NIH Clinical Compound Collection was screened to find inhibitors of the uridine ribohydrolase enzyme required by the parasite to scavenge uracil for its growth. The proton-pump inhibitors omeprazole, pantoprazole, and rabeprazole were identified as inhibitors of this enzyme, with IC_{50} values ranging from 0.3 to 14.5 μM. This suggests a molecular mechanism for the in vitro antitrichomonal activity of these proton-pump inhibitors, and may provide important insights toward structure-based drug design.

Trichomoniasis is the most prevalent non-viral sexually transmitted disease. The causative agent is the parasitic protozoan *Trichomonas vaginalis*. It affects an estimated 170 million people worldwide with as many as 85% of cases occurring in developing nations.[1] More than 1 million new cases are reported each year in the United States, with infection prevalence estimated to be almost 4 million.[2] Although clinical manifestations of trichomoniasis infections are typically mild, the immune system can be concomitantly compromised. Individuals with trichomonal infections have a higher susceptibility to more serious conditions such as cervical cancer, HIV-1, and pelvic inflammatory disease.[3,4] More recently, trichomonal infection has also been associated with prostate cancer and benign prostatic hyperplasia.[5,6] Trichomoniasis is typically treated with 5-nitroimidazole drugs such as metronidazole.[3,7] The compounds themselves are inactive, but are anaerobically reduced by redox enzymes in the parasitic hydrogenosome. Pyruvate-ferredoxin oxidoreductase reduces the nitro group forming toxic nitro radical anions which target thymine and adenine residues in the pathogen's DNA. Cleavage of DNA results in parasitic death within eight hours.[3,7] Resistance to metronidazole and related drugs is increasing, with an estimated 5% of trichomoniasis clinical cases resulting from *T. vaginalis* strains with some resistance.[7]

T. vaginalis is an obligate parasite in that it is incapable of the de novo synthesis of purine[8] and pyrimidine rings.[9,10] It must scavenge nucleosides from host cells and then use salvage pathway enzymes to obtain the nucleobases. Once acquired, the parasite relies on the activity of these enzymes to metabolize the nucleosides. Salvage pathway enzymes are thus potential targets for therapeutic intervention. One such enzyme is uridine nucleoside ribohydrolase (UNH),[11] a fundamental constituent in the uridine salvage pathway. UNH is responsible for cleaving the N-glycosidic bond between uracil and ribose. The identification of UNH inhibitors may lead to novel antitrichomonal agents that are mechanistically distinct from current therapies. An ^{19}F NMR-based activity assay, utilizing 5-fluorouridine in place of uridine, was thus developed and used to screen the NIH Clinical Compound Collection for UNH inhibitors.

An NMR-based assay was developed for monitoring the UNH enzyme reaction in order to avoid many complications associated with screening compounds at high concentrations by other methods.[12] While ^{1}H NMR was sufficient to monitor the hydrolysis of the natural substrate uridine, monitoring the hydrolysis of 5-fluorouridine with ^{19}F NMR was found to be superior for several reasons. First, the ^{19}F NMR spectra are much simpler since they are not compromised by the presence of test compound resonances or a large signal from the non-deuterated DMSO used to plate the NIH Clinical Collection. Second, the K_m values for uridine and 5-fluorouridine were determined to be 54 μM and 15 μM, respectively, indicating that 5-fluorouridine will provide a lower

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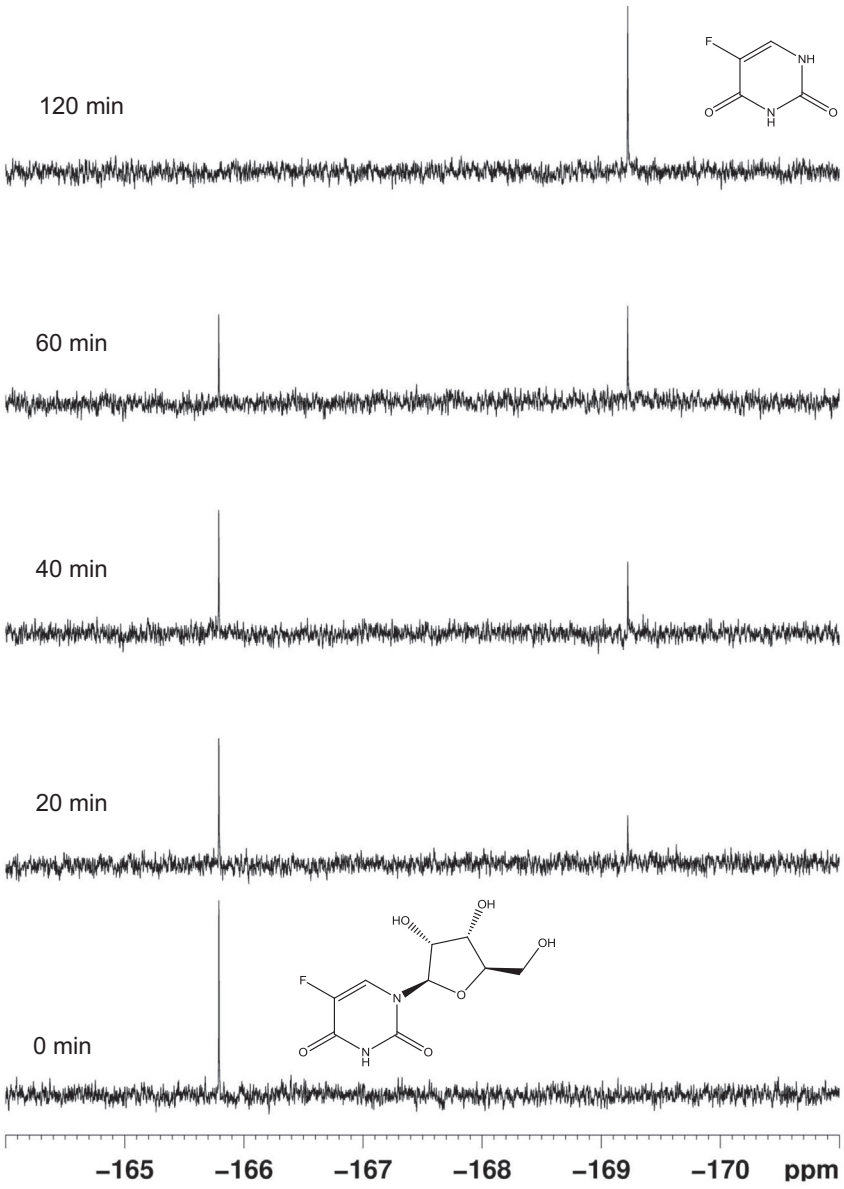

Figure 1. Time course of the ^{19}F NMR-based activity assay for UNH. Structures of the 5-fluorouridine substrate and 5-fluorouracil product are indicated on the 0 min and 120 min spectra, respectively.

concentration hit threshold compared to uridine.[13] The time course for the UNH enzyme assay is shown in Figure 1. Each ^{19}F NMR spectrum[14] was acquired on a separate reaction sample that was quenched at the time indicated. The 0 min spectrum was re-collected after two days and again after two weeks (not shown). No product signal was observed in either of these spectra indicating that samples can be run in parallel and then queued for sequential data collection. Importantly, the ^{19}F chemical shifts for the 5-fluorouridine substrate (−165.8 ppm) and 5-fluorouracil product (−169.2 ppm) are clearly resolved allowing either or both to be used to monitor the reaction. The 40 min time point was chosen for screening assays and IC_{50} value determinations.

Compounds tested for UNH inhibition were a subset of the NIH Clinical Collection and NIH Clinical Collection 2, supplied plated as 10 mM DMSO solutions by Evotec (US) Inc. In order to increase throughput they were screened at a concentration of 50 μM in mixtures of three using a total assay volume of 600 μL. This compression and concentration resulted in a final DMSO content of 1.5%. Initial screens were carried out in batches.[15] Compounds with predicted water solubilities less than 50 μM were excluded from screening with the exception of selected compounds with borderline predicted solubilities. In total, 573 of the 727 compounds in the NIH Clinical Collection were screened (Supplementary Tables 1 and 2). As an example of typical data, Figure 2 shows the screening results for the compounds in plate NGP-104-06. This plate contained 30 compounds (out of 46) that met the solubility criteria and thus comprised 10 mixtures (M1–M10). Compared to the 40 min control spectrum, significant inhibition was observed for mixtures M1 and M2. Both of these mixtures were then subsequently deconvoluted to determine if the inhibition could be attributed to a single compound in the mixture. Figure 3 shows the deconvolution spectra for mixture M2. The inhibitory activity was found to reside in well H2 which corresponds to the proton-pump inhibitor rabeprazole. A total of 23 compounds, corresponding to 4.0% of those screened, were determined to significantly inhibit UNH (Supplemental Table 3). In addition to rabeprazole, the proton-pump inhibitors omeprazole and pantoprazole were also identified as UNH inhibitors. IC_{50} values were then determined

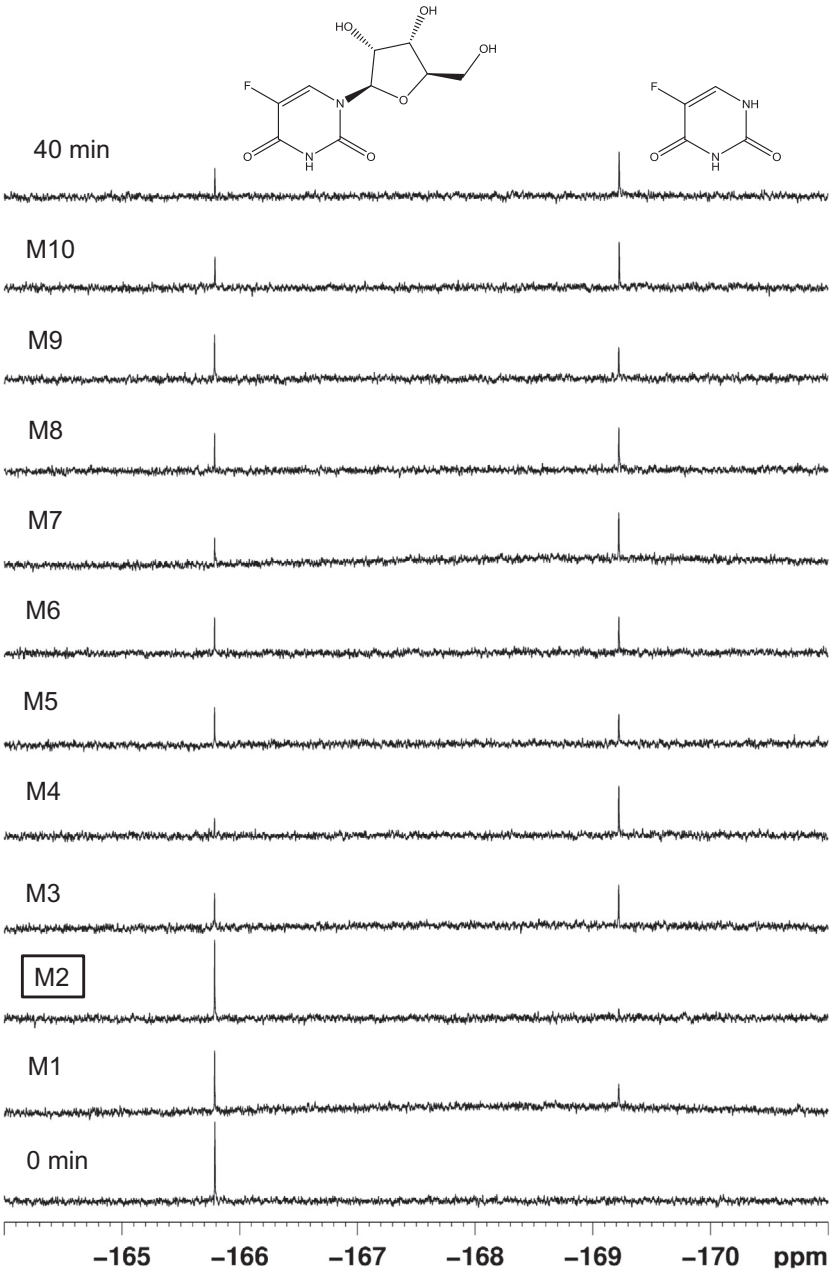

Figure 2. Control (0 min and 40 min) and mixture (M1–M10) ^{19}F NMR data sets for NIH Clinical Collection plate NGP-104-06.

for these compounds using solids obtained from Sigma–Aldrich.[16] The structures and corresponding NMR IC_{50} values for these compounds are shown in Figure 4.

The three proton-pump inhibitor drugs identified here as UNH inhibitors have been previously reported to possess in vitro antiprotozoal activity against *T. vaginalis*, with all three compounds having submicromolar in vitro IC_{50} values.[17] The benzimidazole moiety of the proton-pump inhibitors is a common scaffold found in other antiprotozoal compounds. These findings have led to the synthesis of a series of benzimidazole derivatives that are even more potent than the parent compounds and, even in the absence of information regarding the molecular target, provide useful structure–activity relationships for medicinal chemistry efforts.[18] A second in vitro study involving the testing of 1040 compounds in the US Drug Collection Library has also reported omeprazole and rabeprazole to have modest but measurable *T. vaginalis* growth inhibition.[19] While the mechanism of action for the antiprotozoal activity of these compounds has not been reported, the data described here suggests one possible molecular mechanism. A reduction in the activity of UNH would reduce the parasite's ability to obtain uracil and thus prevent its growth and replication.

The mechanism of action of the 'prazole' compounds with respect to UNH is likely to be significantly different from how they function as proton-pump inhibitors. In the latter, the compounds are first activated in the highly acidic environment of parietal cells to form reactive thiophiles that then bind irreversibly to cysteine sulfhydryl groups of the H^+/K^+-ATPase thereby inhibiting its activity.[20] Prodrug activation is at least two orders of magnitude slower at the pH of 6.5 used here in the UNH assay, and would be negligible during the reaction time period.[21] Further, comparisons to other nucleoside hydrolases indicates the absence of cysteine residues near the UNH active site.[22]

Five of the 23 hits are from a second structural class, namely the calcium channel blockers represented by amlodipine, nicardipine,

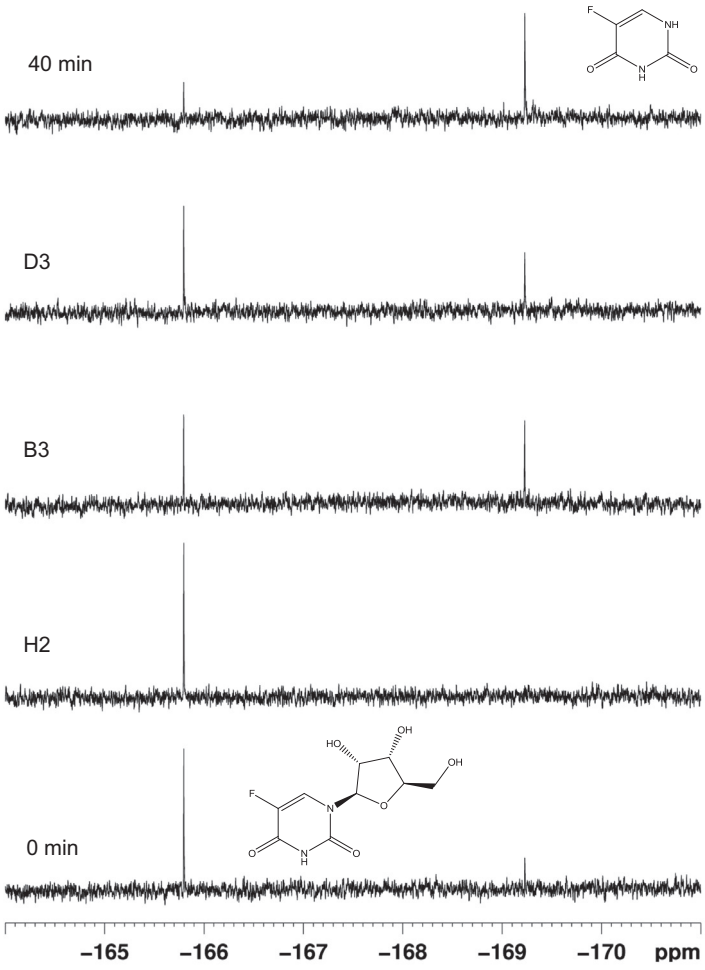

Figure 3. Control and deconvolution ^{19}F NMR data sets for mixture 2 from NIH Clinical Collection plate NGP-104-06. Wells H2, B3, and D3 contain the compounds rabeprazole, irinotecan, and desloratadine, respectively.

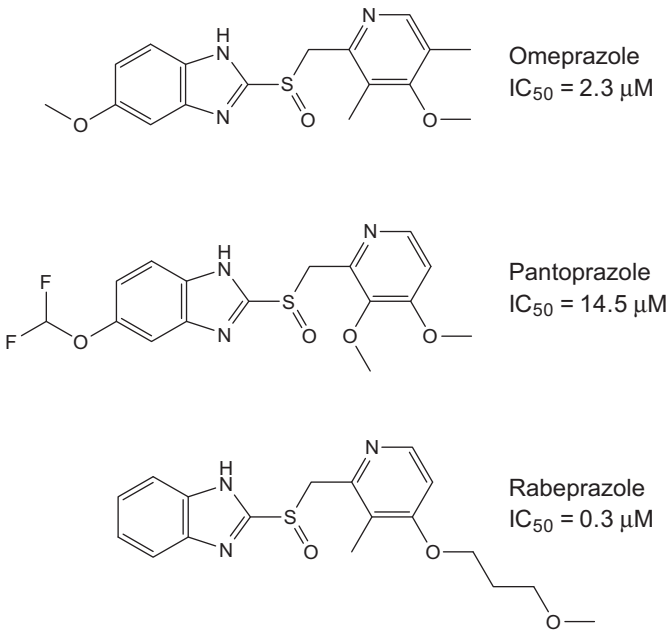

Figure 4. Structures and NMR IC_{50} values for the proton-pump inhibitors identified as UNH inhibitors.

nifedipine, nisoldipine, and nitrendipine. These light-sensitive compounds do not possess in vitro activity[19] and have weaker inhibition in the NMR assay when retested using freshly-made solutions from solids. Studies are currently in progress to determine if the UNH activity observed in the original screen arises from the parent compounds or from breakdown products that may form during compound plating or storage.

The NIH Clinical Collection was chosen as the starting point for this work because the compounds are highly drug-like with known safety profiles, have reasonable diversity that targets a wide range of therapeutic indications, and are readily available as part of the NIH Molecular Libraries Roadmap Initiative. The compounds are inherently bioactive and may provide starting points for medicinal chemistry optimization or structure-based drug design.[23] Future research will explore both of these areas. Smaller fragments of the 'prazole' series of compounds are being tested to determine their ligand efficiencies along with related compounds derived from substructure searches. Molecular modeling and multinuclear NMR spectroscopy are also being used to explore the 'prazole' binding site on UNH.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.01.014. These data include MOL files and InChiKeys of the most important compounds described in this article.

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14. NMR data sets were collected on a Bruker AvanceIII 500 MHz spectrometer equipped with a BBFO probe and a SampleXpress. For the initial screens, 1D $^{19}F\{^{1}H\}$ spectra were acquired using 256 scans and a sweep width of 9398 Hz. The acquisition and relaxation delay times of 0.872 s and 4.0 s, respectively, resulted in a total acquisition time of 20 min per spectrum. For IC_{50} value determinations, 1D $^{19}F\{^{1}H\}$ spectra were acquired using 1024 scans in order to improve the quantitation of remaining substrate.
15. Prior to screening, plates were thawed to room temperature and mixed by gentle rocking for two hours. A stock solution was made first containing 50 mM potassium phosphate and 0.3 M KCl at pH 6.5, 10% $^{2}H_2O$, and 80 nM UNH. Test compounds were then added to aliquots of the stock solution and mixed thoroughly. Reactions were initiated with the addition of 5-fluorouridine to a final concentration of 50 μM. Reactions were quenched with 10 μL of 1.5 M HCl after 40 min. Solutions were then transferred to Norell 502 NMR tubes and queued for data collection. Control samples were prepared and assayed similarly substituting DMSO in place of compounds.
16. Compounds were dissolved and diluted accordingly with DMSO to provide final assay concentrations ranging from 200 μM to 0.04 μM.
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