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Adenosine/guanosine preferring nucleoside ribohydrolase is a distinct, druggable antitrichomonal target

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ABSTRACT

Nucleoside salvage pathway enzymes used by *Trichomonas vaginalis* are distinct from the pathway involved in activation of existing 5-nitroimidazole drugs. They thus represent excellent targets for developing novel, mechanism-based antitrichomonal agents. The purine-specific adenosine/guanosine preferring ribohydrolase (AGNH) was screened against the NIH Clinical Collection to assess its druggability. Eight compounds, including five flavonoids, were identified with IC_{50} values $\leqslant 10$ μM and confirmed in counter screens run in the presence of detergent. The inhibitors are structurally distinct from inhibitors of the pyrimidine-specific uridine ribohydrolase (UNH) thus indicating that AGNH is a distinct, druggable target from UNH.

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Trichomoniasis is the most prevalent non-viral sexually transmitted disease, with the majority of cases occurring in developing nations.[1,2] However, more than 1 million new cases are reported each year in the United States, with infection prevalence estimated to be almost 4 million.[3] Clinical manifestations of infection are typically mild, but the immune system can be concomitantly compromised. Trichomonal infections predispose women to higher susceptibility for more serious conditions such as cervical cancer, HIV-1, and pelvic inflammatory disease.[4,5] In men, trichomonal infection has also been associated with prostate cancer and benign prostatic hyperplasia.[6,7] The causative agent is the parasitic protozoan *Trichomonas vaginalis*. The 5-nitroimidazole class of therapeutic agents in use for the past 50 years requires activation in the parasite's hydrogenosome to form cytotoxic nitro radical anions.[4,8] Resistance to these drugs has increased markedly in recent years indicating the need for new therapies with novel mechanisms of action.[8–10] *T. vaginalis* is an obligate parasite in that it is incapable of the de novo synthesis of purine[11] and pyrimidine rings.[12,13] It must scavenge nucleosides from host cells and then use salvage pathway enzymes to obtain the nucleobases. Nucleoside salvage pathway enzymes used by *T. vaginalis* are distinct from the pathway involved in activation of the 5-nitroimidazole drugs, and do not have mammalian counterparts. They thus represent excellent targets for developing mechanism-based antitrichomonal agents. The first step in this pathway is the hydrolysis of nucleosides to release the nucleobases. The pyrimidine-specific uridine ribohydrolase (TVAG_092730)[14] and the purine-specific adenosine/guanosine preferring ribohydrolase (TVAG_213720)[14] have been characterized enzymatically.[15,16] The two enzymes have markedly contrasting substrate specificities. AGNH efficiently hydrolyzes adenosine and guanosine and has marginal activity toward inosine, but does not hydrolyze cytidine or uridine. UNH efficiently hydrolyzes uridine, has marginal activity toward cytidine, but does not hydrolzye adenosine, guanosine, or inosine. Given these substrate specificities, we hypothesize that AGNH and UNH represent distinct, druggable targets. We previously tested the druggability of UNH by developing an ^{19}F NMR-based activity assay and screening the NIH Clinical Collection for inhibitors. A series of proton-pump inhibitors were identified as μM inhibitors,[17] and are currently being further explored using medicinal chemistry. Here we report the development and application of an NMR-based activity assay to screen AGNH against the NIH Clinical Collection.

By analogy to the UNH screening assay, we initially tried to monitor the AGNH reaction using 2-fluoroadenosine as the substrate. However, this compound is a surprisingly poor substrate with the reaction taking days to show significant progress as shown in Figure 1A. Since other fluorinated analogs of either adenosine or guanosine are not commercially available, we instead developed a ^{1}H NMR-based activity assay using adenosine as the substrate (the K_m value for adenosine is 54 μM).[18] The reaction time course shown in Figure 1B is complete in about an hour.

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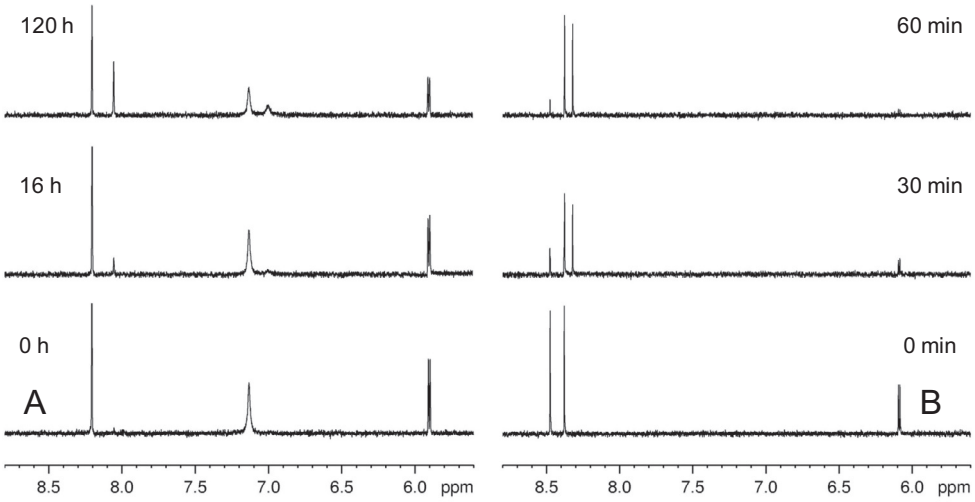

Figure 1. Time courses of the ^{1}H NMR-based activity assay for AGNH using (A) 2-fluoroadenosine and (B) adenosine as substrate.

Compounds tested for AGNH inhibition were a high-solubility subset[20] of the NIH Clinical Collection and NIH Clinical Collection 2, supplied plated as 10 mM DMSO solutions by Evotec (US) Inc.[21] A total of 573 of the 727 compounds were screened (Supplementary Tables 1 and 2) in mixtures of three.[22] A representative example of screening assay data is shown in Figure 2 for plate NGP-104-02. Control spectra in the absence of test compounds with reaction times of 0 and 40 min are shown along with spectra for 8 mixtures (M1–M8). Despite the presence of numerous ^{1}H resonances from the test compounds, substrate (6.10 and 8.48 ppm) and/or product (8.33 ppm) resonances are clearly resolved in all spectra. Compared to the 40 min control spectrum, significant inhibition was observed for mixture M2. Deconvolution of the mixture by testing each of the three components individually identified the active constituent to be (±)-taxifolin. A total of 20 compounds, corresponding to 3.5% of those screened, were determined to have greater than 50% inhibition at 50 μM (Supplementary Table 3). Compounds that exhibited the strongest inhibition in the screening assays were then obtained commercially, structurally validated using 2D ^{1}H–^{13}C NMR, and tested to determine their IC_{50} values.[23] The dose-dependent assay spectra for (±)-taxifolin are shown in Figure 3. The IC_{50} value was determined to be 5.9 ± 2.6 μM.

A total of eight compounds, including five flavonoids, were found to have IC_{50} values $\leqslant$10 μM as listed in Table 1. Since the flavonoids in particular are known to be pan assay interference compounds because of their hydrophobicity and/or catechol moiety,[24,25] IC_{50} values were also determined in the presence of 0.01% Triton X-100. If a compound no longer inhibits, or its IC_{50} value shifts significantly in the presence of detergent, then non-specific inhibition is suspected.[26] For all compounds tested the IC_{50} values remained within a factor of two in the presence of Triton X-100 within experimental error. This indicates that the inhibition is not aggregation-based. Further evidence for the lack of aggregation is observed in Figure 3. As the concentration of

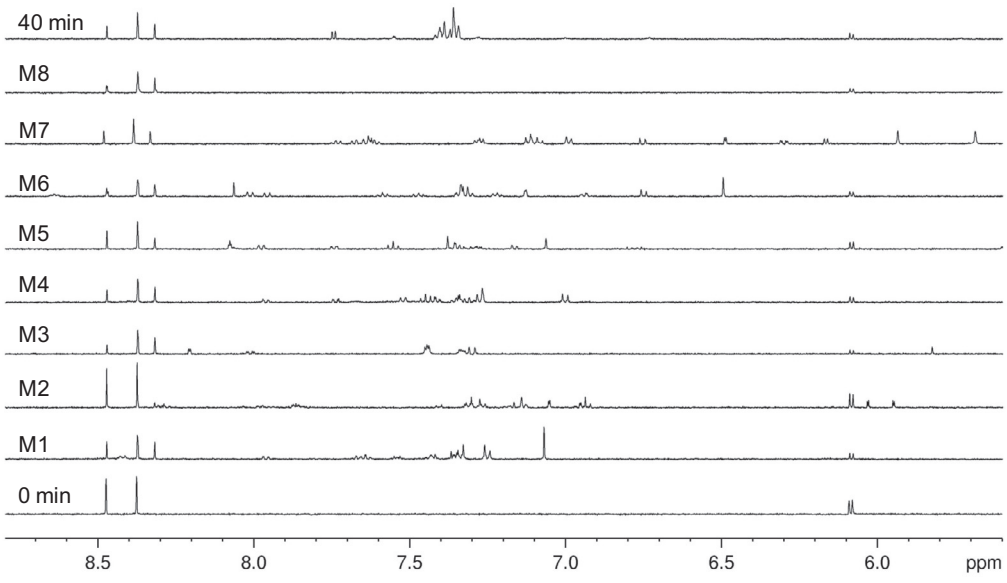

Figure 2. Control (0 min and 40 min) and selected mixture (M1–M8) ^{1}H NMR data sets for NIH Clinical Collection plate NGP-104-02.

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