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Development of new 5-nitroimdazole drugs against intestinal *Tritrichomonas foetus* infections

A Thesis submitted in partial satisfaction for the requirements for the degree of

Master of Science

in

Biology

by

Jeffrey Liu

Committee in charge:

Professor Lars Eckmann, Chair
Professor Kathleen French, Co-Chair
Professor Amy Kiger

2015

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The Thesis of Jeffrey Liu is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

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Chair

University of California, San Diego
2015

DEDICATION

This thesis is dedicated to Tiffany, Robert, and Emily Liu

for their constant love and support.

Without them this academic and intellectual pursuit would never have been possible.

Thank you.

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LIST OF ABBREVIATIONS

Mz	Metronidazole
Rz	Ronidazole
STAT KO	STAT Knockout
STAT WT	STAT Wildtype
5-NI	5- Nitroimidazole
H&E	Hematoxylin & eosin staining
EC_{50}	Effective concentration of a compound to eliminate 50% of the population
GI	Gastrointestinal

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Thank you all in the Eckmann lab, my work that I did as an undergraduate and as a graduate student wouldn't have been possible without the support and motivation of this wonderful group.

.

ABSTRACT OF THE THESIS

Development of new 5-nitroimdazole drugs

against intestinal *Tritrichomonas foetus* infections

by

Jeffrey Liu

Master of Science in Biology

University of California, San Diego, 2015

Professor Lars Eckmann, Chair
Professor Kathleen French, Co-Chair

Tritrichomonas foetus is the causative agent in intestinal feline infections. The current treatment, ronidazole (Rz), is the only 5-nitroimidazole (5-NI) drug shown to have an effect against *T. foetus;* however it has been observed to be less than 70% effective. We, therefore, aimed to develop upon compounds that had a more potent

efficacy against clinically isolated strains of *T. foetus*. In doing so, we screened a library of 630 5-NI compounds, 52 (8%) were found to be more effective than Rz in multiple strains *in vitro*. We developed a novel murine model of *T. foetus* infection to expand upon the efficacy of the compounds under *in vivo* conditions. Elucidating various parameters, antibiotic conditioning was observed to be an effective method in generating a sustainable *T. foetus* colonization. The infection was characterized by a high population of *T. foetus* within the cecum as well as histological evidence showing a host inflammatory response. Selected compounds which showed superior activity *in vitro*; were examined in our newly established *in vivo* model, providing a useful tool in understanding the pharmacokinetics within the host. One compound demonstrated effective capabilities in reducing and inhibiting the growth of *T. foetus*. This compound can be our lead for further development as a potential chemotherapeutic drug against intestinal *T. foetus* infections.

Introduction

Trichomonaisis is an infectious disease caused by parasitic protozoans collectively known as trichomonads (Schwebke and Burgess, 2004). These aerotolerant anaerobic parasites infect a wide range of species, from humans to cats. Trichomonaisis in humans is caused by *Trichomonas vagnalis*, the most common non-viral sexually transmitted infection with around 170 million cases a year worldwide (Cotch *et al.* 1997). Symptoms associated with *T. vaginalis* infection such as pruritus, vaginitis, and vulvitis, are similar to those seen in the bovine infection with another trichomonad species, *Tritrichomonas foetus* (Maritz *et al.* 2014). This relative of *T. vaginalis* is also a sexually transmitted infection; the infection persists in both humans and cattle due to the asymptomatic nature of the infection (Davidson, 2009). Passing from an asymptomatic infected bull to many cows, this spread of infection can devastate a herd of cattle due to the aggressiveness of the *T .foetus* infection (Felleisen, 1999). However, in symptomatic bulls the sperm motility is negatively affected due to the cytotoxic products released from *T. foetus* metabolism, therefore leading to poor breeding seasons (Riberio *et al.* 2010) (Midlej *et al.* 2009). This bovine parasite is also capable of traveling up the vaginal canal, attacking the cervix and moving into the uterine wall as well as the uterine horns (Agnew *et al.* 2008). In symptomatic infected cows, a clinical manifestation of a *T .foetus* infection is pyometra, a uterine infection, which can result in low calf birth weights, spontaneous abortions, and infertility (Midlej *et al.* 2009). *T. foetus* infection can cost a cattle farm around 144 dollars per cow, because the infection can be recurrent, undetectable or even resistant to the treatment (Davidson, 2009). This can lead to increased cost per cow infected with

T .foetus. The national economic impact of *T. foetus* infections per year averages around 650 million dollars (Davidson, 2009).

Multiple studies have been done to understand the pathogenicity as well as the chemotherapeutic potential of various treatment options. However, in *T. vaginalis* studies it was observed that while a small animal model was possible, this required several manipulations such as estrus synchronization, introduction of certain bacteria, and antibiotic pretreatment (Cobo *et al.* 2011). By comparison, the bovine *T. foetus* strains were able to maintain high levels of infection in BALB/c mice (Van Andel *et al.* 1996), thus providing a viable method for mouse model generation in furthering the studies of compounds and infection profiles (Cudmore *et al.* 2004).

Tritrichomonads also infect domestic cats, as first demonstrated by veterinary clinicians in 1996. With similar morphological structures, the parasite was determined to be a form of *T. foetus*, however its mode and site of infection are different from that of the species that was typically observed in the urogential tracts of cattle (Slapeta *et al*. 2012). The jump between species, from bovine to feline, is still unclear to veterinarians, although recent genetic analysis of the two species has shown that the tritrichomonad that infects the intestine of cats is a separate species from the tritrichomonads that infect the reproductive tract of cattle. This distinction between the two species has not been fully recognized among veterinarians. According to recent studies, cats that are capable of being infected by *T. foetus* are not specific to any age, type of cat, or sex, suggesting that no one group of cats is more susceptible to being infected with *T. foetus* (Gruffydd-Jones *et al.* 2013). *T. foetus* infections in cats, unlike those seen in cattle, are localized in the large intestine instead of the reproductive tracts (Yaeger and Gookin, 2005). Due to the

aggressive parasitic nature of *T. foetus*, it remains a viable parasite in the host despite apparently strong host defenses (Vilela and Benchimol, 2011). Current studies have shown that *T. foetus* uses a variety of routes to remain a viable parasite: feeding off of the metabolites from host cells, actively attacking and inducing apoptosis in host cells, or invading further into the mucosa and metabolizing extracellular proteins (Lockwood *et al.* 1984). These studies have also suggested that *T. foetus* is an opportunistic parasite, feeding off of any available extracellular substances. In order to induce apoptosis in host cells, *T. foetus* binds to the surface of the cell either by using the axostyle or by a suction cup mechanism similar to that seen in *Giardia* (Tolbert *et al.* 2013). After binding to the host cells, the parasite forces cytotoxic molecules into the host cell and this causes the host cell to lyse and release molecules that are then metabolized by the parasite. This is only one mechanism that the parasite uses, since *T. foetus* has been seen to induce cytotoxicity in not only epithelial cells but also fibroblasts and myocytes (Vilela and Benchimol, 2012). Placed under *in vitro* conditions, *T. foetus* has been observed to also metabolize keratin, an extracellular protein, by endocytosis (Vilela and Benchimol, 2012). Intriguingly, histological analysis of cat infections has shown that the parasite tends to remain on the surface of the mucosa; rarely did the parasite go below the surface of the mucosa. Furthermore, the tritrichomonads were rarely observed to go below the surface of the colonic crypts, when presented with the conditions that mimic the deep mucosa environment (Yaeger and Gookin, 2005). Once the parasite binds to the host's surface of cells, the resulting inflammatory signals cause the large-bowel diarrhea in felines which is commonly associated with *T.foetus* infections (Woodruff *et al,* 2011). The exact mechanisms of how the host develops chronic large bowel diarrhea is still unknown

(Mostegl *et al.* 2012). Symptoms such as colitis, which result in malodorous bloody or mucus stools, are often only seen in kittens, cats younger than one year of age (Gookin *et al.* 2004). Though older cats may be infected, they do not commonly develop symptoms that are associated with *T. foetus* infections (Mutwiri and Corbell, 1998). As a result, they remain untreated, which leads to older cats becoming a source of infection. This increases the probability that younger cats in high population density areas, such as catteries, shelters and houses with multiple cats, to also contract the infection (Gookin *et al.* 2004). Once an older cat, with no symptoms of infection, uses the litter box, the remnant feces contains living and viable parasites. Another cat that uses the same litter box can contract the infection by stepping in the feces and then later grooming itself, ingests the parasite and thus contracts the infection (Van der Saag *et al.* 2011). With this undetectable infection persisting in older cats, the current estimate of infected cats in the US is around 10 -15% of the entire population of cats (Gruffydd-Jones *et al.* 2013).

In order to treat trichomonaisis caused by *T. vaginalis* and *T. foetus*, both are prescribed a 5-nitroimidazole drug known as metronidazole (Mz) (Cudmore *et al.* 2004). While this clears the infection in both humans and cattle, depending on the dosage regimen, this nitroimidazole compound is not effective against the intestinal *T. foetus* infections in felines (Cunnigham *et al.* 1994). Treating cats with other drugs that have been used in similar infections, such as fenbendazole, paromomycin, tinidazole furazolidone and metronidazole, relieve the symptom of diarrhea, but these drugs are ineffective in clearing the infection (Gookin *et al.* 2007). In veterinary clinical settings, intestinal *T. foetus* infections in felines are commonly misdiagnosed for *Giardia*, due to the fact that under a light microscope the two pathogens have morphological similarities

(Gookin *et al.* 2004). In felines, metronidazole is prescribed for the treatment of giardiasis, infections caused by *Giardia* (Valdez *et al.* 2009). However, this misdiagnosis leads to the incorrect prescription, which relieves infection symptoms, but does not eradicate the pathogen (Gookin *et al.* 2006). Currently there is no highly effective drug to treat intestinal *T. foetus* infections, although veterinarians routinely employ another 5-nitroimidazole (5-NI) compound known as Rz (Gookin *et al.* 2006).

The efficacy of these compounds is owed in part to the cellular structure of the protozoan. Trichomonads lack mitochrondria, so instead of relying on these organelle powerhouses for ATP, trichomonads utilize the glycolytic pathway by using organelles known as hydrogenosomes (Kulda, 1999). The main function of these organelles is to oxidatively decarboxylate pyruvate, coupled with ATP production and a ferredoxin-mediated electron transport, resulting in the production of molecular hydrogen (Lloyd et al. 1979). The key enzyme in the metabolism of pyruvate in hydrogenosomes is pyruvate ferredoxin oxidoreductase (PFOR), which converts pyruvate into acetyl-CoA. PFOR also functions in the reoxidation of NADH, converting it into NAD, which can then function as an electron carrier (Kulda, 1999). This metabolic pathway is shared equally between *T. vaginalis* and *T. foetus*, with no observable differences in hydrogensomal metabolism. The 5-NI compounds make use of these pathways in the trichomonads, entering both the cell and the organelle by simple diffusion (Müller and Gorrell, 1983). Competing with native electron acceptors in the parasite, molecular hydrogen production is halted and the ferredoxin transport of electrons is directed to the 5-NI compound (Ryley, 1954). This reduction of the drug converts it into a toxic intermediate, generating an anion radical, which may target the cell's DNA and other critical molecules leading to cellular death

(Kulda, 1999). However, the complete mechanism of the compound's action is not fully known. Clinically, human and animal strains of trichomonads have been observed to develop a resistance to the current treatment options. Studies suggest that there is a significant decrease in the expression of PFOR, thus decreasing trichomonad response to current therapeutic methods (Kulda, 1999). This reduced response results in current clinical observations of the increased trichomonad antimicrobial drug resistance development.

While Rz is not currently FDA approved in animals, the drug has been shown to be moderately effective in treating *T.foetus* intestinal infections in cats. The current dosage regimen prescribed by veterinarians for *T. foetus* infections is Rz at 30 – 50 mg/kg once a day for 14 days (LeVine, 2011). This regimen is usually only prescribed when *T. foetus* infections are confirmed and after obtaining owner consent. Even so, the prescription is monitored heavily due to the neurotoxicity potential of Rz (Xenoulis *et al.* 2013). Signs of neurotoxicity include lethargy, ataxia, trembling of the extremities, facial tremors, agitation and hyperesthesia (Gruffydd-Jones *et al.* 2013). Although this an important factor to consider with Rz, another important aspect to consider is the drug's effectiveness. Its toxicity is due in part to the dosage regimen that is required to completely clear the host of this parasite. A higher plasma drug concentration might be expected to increase the chances of the host developing neurotoxic symptoms (Upcroft *et al.* 2006). Current studies have also suggested that more information is needed to examine the efficacy of Rz at lower doses and less frequent dosages episodes, in order to relieve the development of neurological symptoms. Furthermore, a retrospective study found that out of 45 cats only 29 (64%) had a good response to the current therapy (Xenoulis *et al.* 2013). Sixteen of the

45 (36%) had either no or partial response to the Rz with some subjects relapsing after the therapy was discontinued, suggesting that Rz was not an effective therapy for treating *T. foetus* infection (Xenoulis *et al.* 2013. Thus an area for developing and testing new 5-NI compounds that are more effective against the protozoan, while requiring a less frequent dosage regimen, is necessary in order to maintain a longer and more efficacious treatment of *T. foetus* intestinal infections in cats (Miyamoto *et al.* 2013).

New 5-NI compounds, such as Mz, have proven to be the more effective than existing drugs of this class, (Miyamoto *et al.* 2013). These compounds have been synthesized by click chemistry, a coupling reaction between a reactive azide, a 5-NI core, and various alkynes (Miyamoto *et al.* 2013). Screening these compounds against various feline strains of clinically isolates of intestinal *T. foetus* is a necessary step in selecting certain compounds for further drug development. Similar methods were applied to screening various strains of *Giardia*, eventually developing a small library of compounds that were more efficacious than the current pharmaceutical options (Tejman-Yarden *et al.* 2011).

Drug development usually requires a small animal model of infection, but such a model does not exist for intestinal *T. foetus* infections. Consequently, the aims of the present study were to test new 5-NI compounds against multiple strains of tritrichomonads whilst comparing these values against those set for the currently used compounds, Mz and Rz. The data gathered from these screens were used to select several new potent compounds, which were then tested for efficacy in a newly developed murine model of intestinal *T. foetus* infection.

Materials and Methods

Strains of trichomonads

One cow derived strain of *T. foetus*, D1, and several cat strains of *T. foetus* were used in these studies. The *T. foetus* D1 strain was isolated from a cow kindly given by Dr. Corbeil at the University of California, San Diego. The intestinal *T. foetus* strains 364287+, 347058+, 2002-96+++ were kindly given by Dr. Land from Pacific University. All the tritrichmonad strains were grown axenically in anerobic conditions at 37ºC in trypticase-yeast extract maltose (TYM) medium, supplemented with an iron solution and 10% bovine serum and adjusted to a pH of 7.0. All cultures were grown, stored, and frozen in Cryo Media freezing solution, 8:1:1 ratio of TYM media, horse serum, and DMSO, and placed in -80ºC freezers.

Animals

C57 (5-13 weeks) and STAT KO/WT (7-16 weeks) mice maintained under specific pathogen-free conditions, were used for these studies. The age of the animals did not influence the outcome of the severity of the infection. The animals were housed together 5 per cage and feces were allowed to remain in the cage. Animals were maintained under specific non-pathogenic conditions. All animal studies were reviewed and approved by the University of California, San Diego, Institutional Animal Care and Use Committee.

Oral inoculation of mice

Tritrichomonads were harvested from in vitro culture samples by centrifugation and resuspended with TYM medium at a concentration of $5x10^6$ tritrichomands / ml of TYM media. Each mouse was inoculated with 200µl of this suspension by oral gavage, bringing to a final total of 10^6 tritrichomonads per mouse. Between each experimental group, mice were given a mixture of antibiotics which was placed in the water source for each cage.

Antibiotic pretreatment of mice for T.foetus intestinal infection

Using neomycin 1mg/ml, vancomycin 0.5 mg/ml, ampicillin 1mg/ml, the antibiotics were placed into the mice's drinking water and given ad libitum. The antibiotics reduced the host's native flora creating a niche that the tritrichomonads were able to fill. Although the initial development of the infection only requires a standard pretreatment regimen of 4 days, in order to sustain the infection at significant levels antibiotic treatment in parallel with the infection was necessary.

Analysis of infection and symptom development

Normal C57 mice were given an antibiotic pretreatment prior to administering the *T. foetus* 346287+ strain at a concentration 10^6 trichomonads/200µl/mouse. Fecal samples were collected from infected animals and weighed in 50 ml tubes. The difference

between in tube mass prior to and after fecal samples were collected was calculated. Five ml of Dulbeco phosphate buffer solution (DPBS) was added to the pellets, which were grinded or vortexed until a suspension was created. Tritrichomonad load was determined by taking 10µl and counting the number of tritrichomonad using a hemocytometer. Within the hemocytometer field, the number of trichomonads counted is related to 1 cell correlates to 10^4 cells / ml of the sample. Proportionally, a larger sample of feces in an infected animal would correlate to a higher tritrichomonad count in the hemocytometer. As a result, after the total trichomonad population in the sample was calculated, this was standardized by dividing the count by the mass of feces collected from each mouse.

Analysis of tritrichomonad distribution and histological disruption

Mice confirmed by the fecal sample collection to be positive for the *T. foetus* infection were euthanized via CO_2 inhalation and cervical dislocation. The following organs were collected: small intestine, cecum, large intestine, liver, spleen and mesenteric lymph nodes. The liver, spleen and mesenteric lymph nodes were placed in 10% formalin for overnight fixation. The small and large intestine were divided in the center and split between proximal and distal sections. Portions of each section were taken along with its contents and placed in DPBS to evaluate the distribution of tritrichomonads in each section of the intestinal tract. The same was done for the cecum. Each section was suspended in 2 ml of DPBS and 10 µl of the suspension was loaded onto a hemocytometer. Counts were done three times to ensure that the sample contained no live tritrichomonads. The rest of the sample was cut along the longitudinal length of the organ

and rolled from the proximal end to the distal end. The samples were dipped, left to air dry, and then placed in 5 ml of 10% formalin overnight. After the overnight dehydration in formalin, the samples were transferred to 70% isopropyl solution for preservation later histological processing. The tissue samples were then fixed and stained following the hematoxylin and eosin (H&E) staining protocol. Detecting the invasive ability of the tritrichomonads was based on determining sites of inflammation via the invasion of white blood cells into the mucosa. Using a Nikon Eclipse 50i to visualize the inflammation, leukocytes were seen invading into the lamina propia disrupting the epithelium and the mucularis mucosa. Visual images were captured and saved as .tif files using the software Nikon ACT-1.

Drug susceptibility screening assay

Drug stocks, synthesized by Dr. Kalisiak at the Scripps Research Institute, (10 mM in dimethyl sulfoxide) of 5-NI derived compounds were diluted in DPBS to 375 µM, to create stock compound plates. From the 375 µM compound stock plate, dilutions were made to dilute the compounds to a 75 µM compound test plate, and 1:3 serial dilutions were made in 40 µl of TYM media, Trichomonas growth media, in 96-well microtiter plates. The final concentrations of the compounds ranged from 20µM to 0.4 nM, covering a 5-$\log_{10}$ range. Ten microliters of each strain, either feline or bovine derived intestinal strain, at $3x10^3$, was placed in each well, translating roughly to $3x10^5$ trichomonads per plate. Each tritrichomonad strain had a different concentration per well based on each strain's baseline response to Mz and Rz. After the tritrichomonads were

added to each plate, they were placed in an incubator at 37ºC under anaerobic conditions (AnaeroPack, Mitsubishi Gas Chemical, Remel, Leneka, KS). Twenty-four hours after challenging the parasites with the compound library, the plates were removed and each well was measured for differing levels of cellular activity, based on ATP levels. This was measured by the concentration of ATP within that well, where the higher the ATP concentrations were, this inferred a healthier population of cells that managed to sustain through the compound challenges (BacTiter-GloMicrobial Cell Viability Assay Promega, Madison, WI). The luminescent signal was measured in a microplate reader (SpectraMax M_2e, Molecular Devices, Sunnyvale, CA). The compound concentration, which reduced *T. foetus* population to 50% compared to other cultures without added compounds (50% effective concentration or EC_{50}) was determined by graphical analysis of the concentration-response curves and expressed as a log value (log EC_{50}). The assays were repeated three times for each compound in order to generate a reliable average with an acceptable standard deviation.

Statistical analysis

EC_{50} data for each compound were analyzed with BioAssay. The log EC_{50} values were then converted to micro molar values and plotted in a table along with the pEC_{50} values with the standard errors. Compounds that had standard deviations greater than 0.50 were analyzed individually for each log EC_{50} values from each experiment. Comparing the experimental groups to the control group in the in vivo drug screening

process, the groups were compared to each other by ANOVA through GraphPad prism software. Bonferroni tests were performed for the groups if the overall $p<0.05$.

Treatment of T. foetus infection with 5-NI compounds

The compounds that were selected to test in vivo in mice against the *T. foetus* intestinal infection were evaluated based on the EC_{50} value of compounds that were more potent than Rz. The top 95 compounds that were more potent in strain 364287+ and D1, were then used to screen against other feline isolated strains, 347058+ and 2002-96+++. After analyzing the data from the latter screened strains, compounds that were more efficacious could be used for further screening. Based on in vivo data from previous studies done on other parasitic pathogens, we selected a few compounds based not only on their past in vivo efficacies on other pathogens, but as well as their greater efficacies when compared to Rz. The 5-NI derived compounds were purified and made safe for animal use, and placed in a suspension of 0.2% hypromellose and each mouse received 2 doses for 3 days at 10 mg /kg of mice. Based on the 10 mg/kg dose regimen, the drug concentration was therefore adjusted to 1 mg of mouse weight / 1 ml of volume inoculated. Each group of mice was separated by the compounds that the mice were given, 3 mice per compound and 5 mice remained as the negative control, receiving only the 0.2% hypromellose solution at the same 10 mg / kg of the mice.

Cages were switched with every fecal sample collection and oral gavage procedure. Fecal samples were collected at day 1 and 2 to monitor the infection. On day 4, the compounds were given to the mice via oral gavage and fecal samples were also

collected. On day 5, fecal samples were collected to monitor each compound's in vivo effects, during the dosage regimen. On day 7, fecal samples were also collected to check the compound's immediate effects after the full treatment regimen was administered to the mice. On day 8, the mice were euthanized via CO_2 inhalation and cervical dislocation, and the cecum was collected and weighed. Each cecum was opened and immersed in 2 ml of DPBS, and vortexed. 10 µl of the suspension was loaded into the hemocytometer and counts were performed. The counts were then calculated to include the volume of the sample and divided by the weight of the cecum.

Results

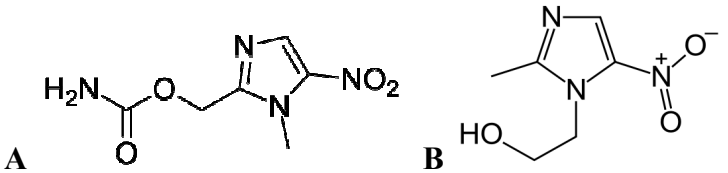
Screening T. foetus strain 346287+ against 5-NI derived compounds

The compound library of 5-NI drugs was originally synthesized by the click reaction (Figure 1D) to yield physiologically stable compounds. With a similar structure to that of Rz, each compound was generated via the click chemistry between a reactive azide and an associated alkyne, the names are defined by the core structures and alkynes reacted to the specified azide (Figure 1C). The compounds were screened against the strains of *T. foetus* and a table of the compound responses' against the various strains was generated. A preliminary screening of the various strains against the common pharmaceutical drugs such as Mz and Rz was done to establish a baseline in vitro log EC_{50} value. Compounds that were unable to reduce the population below 50% were considered to have a log EC_{50} value of -4.7, which converts to 20 μM. Calculating each compound's standard deviation value was done by comparing the log EC_{50} values for each of the experiments for each compound per strain.

The focus of the initial screening process was to screen the entire library of 5-NI compounds against two strains of *T. foetus*, the bovine strain, D1, and a feline intestinal strain, 364287+. This screening process with more than one strain allowed for the selection of compounds that would be efficacious in more than one clinical indication. The log EC_{50} values were converted to the EC_{50} (μM) values and plotted in the cloud plot. Six hundred thirty of the newly derived 5-NI compounds were screened against 364287+, of which 143 compounds, 23%, were found to be more effective than Rz

(Figure 2A). When compared against the *T. foetus* strains, D1 and 364287+, this was based on EC_{50} values less than 0.78 μM and 2.24 μM, respectively. Therefore the lower the EC_{50} value the more effective a compound is said to be. As a result, compounds that had EC_{50} values lower than the EC_{50} value of Rz, in vitro, were selected to be further tested against other intestinal feline strains. The EC_{50} and pEC_{50} values with standard error values placed in a data table for both strain D1 and strain 364287+ are presented in a table (Table S1). The EC_{50} values for each strain were plotted in the cloud plot and selected compounds are highlighted in the light blue square (Figure 2A).

Out of these 143 compounds, 95 were selected for further screening based on the dose response curves each compound had against *T. foetus* 364287+ (Figure 2A). These 95 compounds were further used to screen against other cat derived intestinal strains, 2002-96+++ and 340758+. Due to the relatively high EC_{50} value of strain 340758+ to Rz, 1.42 μM, compounds were further selected from the pool of 95 compounds (Table S2). Fifty-two compounds were found to be more effective, 55%, when compared to the Rz EC_{50} value of strain 340758+. The values of these 630 compounds screened against strain 364287+ and D1 can be found in Table S1 and the values for the selected 95 compounds for further screening against strains 340758+ and 2002-96+++ can be found in Table S2.



Core	Functional group R_1	R_2	R_3
A	N_3	CH_3	H
B	CH_3	N_3	H
C	CH_3	N_3	H
D	CH_3	CH_3	N_3
E	N_3	O	H
F	N_3	N	H

C

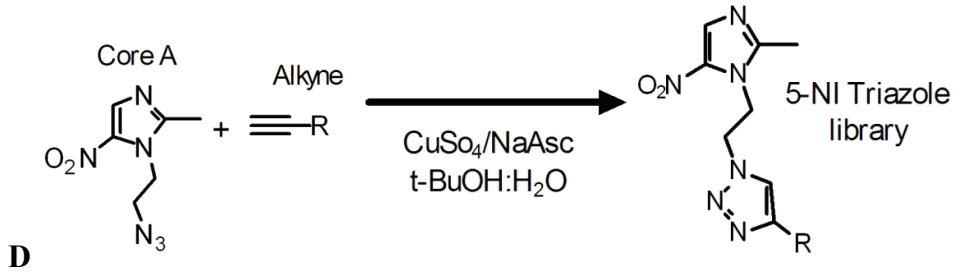


Figure 1. Synthesis of the newly derived 5-nitroimidazole compounds. (A) Structure of Rz. (B) Structure of Metronidazle (C) Six different cores (A-F) were synthesized via a reactive azide. (D) An example of the click chemistry that was used to synthesize the newly derived 5-nitroimidazole compounds.

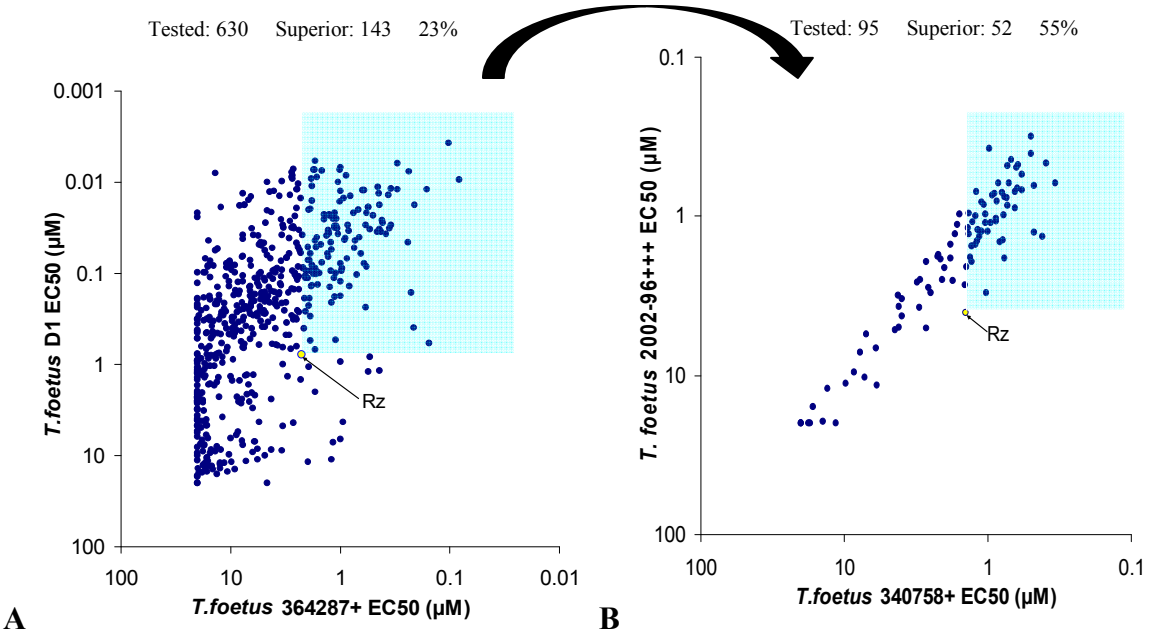


Figure 2. Screening data against various strains of *T. foetus*; D1, 364287+, 340758+, and 2002-96+++. (A) Cloud plot of compound EC_{50} values measured against *T. foetus* strains D1 and 364287 +. Within the highlighted area are compounds that are more effective than Rz, n=143, 23%. The yellow dot represents the strains' responses to Rz. (B) 95 compounds were selected from the group of 143 compounds, screened against *T. foetus* 2002-96+++ and 340758+. 52 compounds, 55%, were found to be more effective in both groups than Rz and a few were selected for *in vivo* screening.

Establishing murine T. foetus infection model

The in vitro screen had suggested that several of the new compounds have superior activity over existing drugs against *T. foetus*. As a next step in drug development, it is usually necessary to test for drug efficacy in a small animal model of infection, but adequate models do not exist for intestinal *T. foetus* infection. In order to develop such a model, I used different infection and conditioning regimens in standard laboratory mice. The general approach was to infect mice orally with tritrichomonads, collect fecal samples, homogenize them in DPBS, and counted live tritrichomonads under a light microscope. Only motile parasites were counted due to the fact that only motile organisms can confer and continue the infection (Figure 3.).

Initial studies showed that normal mice were difficult to infect, so I evaluated the effect of genetic manipulation on the ability of mice to establish infection. Using STAT Cre +, STAT knockout mice (STAT KO), and STAT Cre-, no effects on STAT expression (STAT WT), as the genetic representations was due to the importance of the signaling molecule STAT 3. STAT KO mice had STAT 3 knocked out of the intestinal epithelial cells, an important signaling molecule for maintaining the epithelial cell lining (Akira, 1999). We expected that STAT KO mice would sustain a more robust *T. foetus* infection because STAT KO mice were unable to maintain a healthy intestinal epithelial lining, allowing the tritrichomonads to invade further into the intestinal mucosa. While effectively STAT WT mice would be very similar to the wildtype (WT) mice, genetically, it was expected that this experimental group would have a less robust

infection when compared to the STAT KO mice. However, the infection profiles of both the STAT KO and STAT WT experimental mice were similar (Figure 3A). Both the STAT KO and STAT WT had a similar infection magnitude and there was no significant difference between the two groups during the course of the infection up to Day 4 (Figure 3A). Day 4 of the infection correlated to the tritrichomonads reaching the height of infection (Figure 3B). While STAT KO was expected to have a more robust infection than the STAT WT experimental group, it never exceeded the WT mice on Day 4. There was no significant difference between the two genetic groups, both STAT KO and STAT WT retained the same level of infection on Day 4 (Figure 3A). Without any external manipulations, it was observed that there was no significant difference between the KO and WT groups (Figure 3A).

In other infection models developed for *T. vaginalis* and *G. lambila* murine models, antibiotic pretreatment was necessary to sustain the infection in the host (Cobo *et al.* 2011). As a result, the use of antibiotics prior to infection was the next parameter used in order to develop an infection could be sustained in the intestinal murine *T.foetus* infection model. Infecting mice without the use of an antibiotic pretreatment had an adverse effect on maintaining and detecting significant differences in infection levels between different genetic groups of mice (Figure 3A). The experimental group without antibiotics but were infected with the tritrichomonad (AB^-) had no observable motile tritrichomonads upon direct fecal counts as well as organ counts. This control group was to determine whether an infection would be detectable without the use of antibiotics. Although there were no observable tritrichomonads upon fecal sampling, this did not rule out the fact that our methods were not sensitive enough to view the tritrichomonads. This

calculated value (data not shown) set the threshold for which the infected mice would be considered significant values. During the course of the infection maintaining the mice on the antibiotic regimen helped to sustain the infection above threshold and remain relatively constant above a logarithmic value of 7, 1000000 (Figure 3B). When the pretreatment was included in parallel with the *T. foetus* infection, the infection was able to remain constant and robust through the entirety of the study. This group is stated in the legend as AB^{+}, antibiotic pretreatment and *T. foetus* infection included. Based on data from Figure 3B, the antibiotic pretreatment was shown to be necessary in order to establish the infection, however, whether the pretreatment was sufficient in maintaining a constant and robust infection had yet to be determined. As a result, the next parameter to elucidate was the necessity of using the antibiotics in parallel during the entire study (Figure 3B). Since the antibiotic pretreatment would only affect the native enteric flora of the murine host, an initial antibiotic treatment should remove the native intestinal bacteria, exposing the host epithelium to the tritrichomonads. By only giving the antibiotics in parallel for 7 days with the infection ($A^{+}7$), I expected to find that the levels would remain constant for the entire course of 35 day study, since by Day 7, the infection reaches sustainably high levels (Figure 3B). Compared to the control group, the $A^{+}7$experimental group was able to maintain the infection for 4 weeks at significant levels, however, the infection was not as sustainable when compared to AB+ group. The focus of this experiment was to generate an infection model that was sustainable enough to detect a significant difference in the tritrichomonad population level upon the use of the 5-NI compounds. As a result, the use of antibiotics in parallel with the course of the

infection seemed to be the most effective method for maintaining a sustainable infection (Figure 3B).

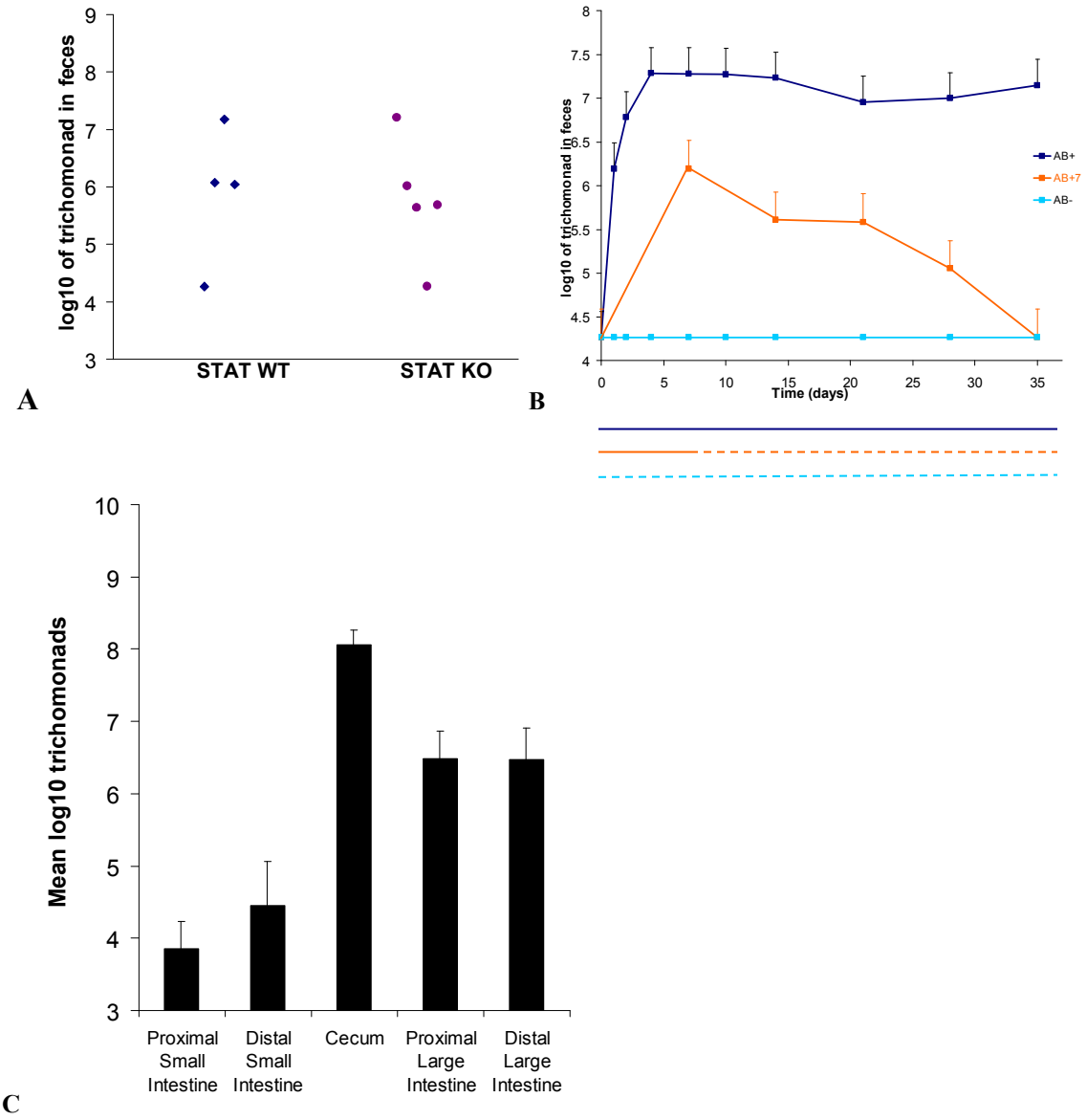


Figure 3. Intestinal *T. foetus* infection profiles in mice models, genetic and wiltype mice. (n>3) mice per group were infected via oral gavage with *T. foetus* 364287+ strain at Day 0. (A) Hemocytometer counts of tritrichomonads in feces, varying on the use of antibiotic pretreatment prior to infection. The parallel antibiotic treatment with the infection is in dark blue. The antibiotic treatment stopped on Day 7 of infection is in orange. The group only exposed to tritrichomonads without antibiotic pretreatment. Length of treatment is marked with lines, solid is on antibiotics and dashed is off antibiotics. (B) Hemocytometer counts of tritrichomonads in feces, testing whether a genetic manipulation would cause any change in infections STAT Cre -/+ (STAT WT/STAT KO), determining whether genetic manipulation affects the sustainability of the infection. (C) Graphical display of *T. foetus* intestinal infection organ distribution.

To gain a better understanding of the localization and nature of the infection, I removed different segments of the GI tract and counted for the presence of tritrichomonads on Day 14 (Figure 3C). The small intestine had the lowest tritrichomonad population, while the cecum had the greatest colonization (Figure 3C). Due to the high colonization of tritrichomonads counted in the cecum and the large intestine, the tritrichomonads were expected to have the highest probability of attacking the host cecal and intestinal epithelial cell lining. In turn, causing a noticeable inflammarory response by the host in the tissue (Figure 3C).

When compared with Day 14 antibiotic treated and tritrichomonad infected mice, there was a visible inflammation of leukocytes into the cecal mucosa, which was the portion of the intestinal tract with the greatest population of trichomonads (Figure 3C). When compared to the control group, there is no noticeable invasion of the leukocytes into the cecum mucosa, an observation associated with *T. foetus* intestinal infections in felines (Figure 4A). Histological samples were done for every portion of the intestinal tract and upon analysis, there was no noticeable host inflammatory response found in the small intestine. The only noticeable infiltration of leukocytes into the lamina propia was found in the cecal tissue. While, the distal end of the cecum contains patches of leukocytes anatomically similar to the Peyer's patches found in the large intestine, the distribution of leukocytes within the cecal mucosa had no distinct shape and was found largely distributed around different regions of the cecum (Figure 4A). As well as observing H&E stained tritrichomonads in association with the cecal epithelium, in slides with a noticeable infiltration of leukocytes (Figure 4B). Each cecum histological slide from infected mice was analyzed and each slide showed a differing distribution of

leukocytes. Compared to the control mice, there was no visible invasion of leukocytes into the lamina propia (Figure 4C). Suggesting there is a positive host-inflammatory response to the presence of tritrichomonads in the cecum.

Although no noticeable host inflammatory response was found in histological slides of the large intestine mucosa, the observation of leukocytes invading the cecal mucosa showed that there was host-parasite interaction in the murine model. Not so in far from the principal host where the host inflammatory response is found in the large intestine. While the response may not be in the large intestine, the observation of a host-inflammatory response applies the model well enough to be an infection model, a necessary tool, in studying the in vivo effects of tritrichomonad infections and compound interaction.

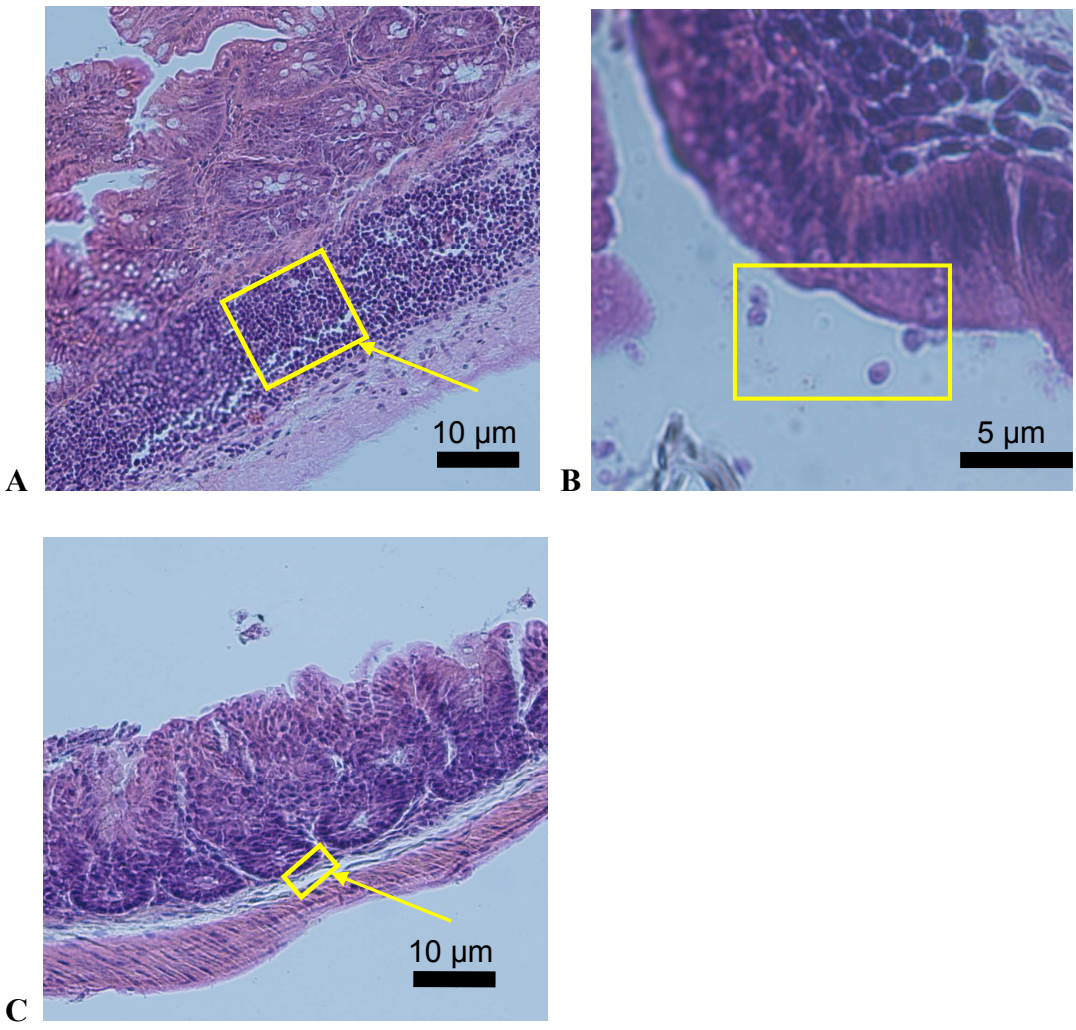


Figure 4. Histology of intestinal *T. foetus* infections in C57 in murine models on Day 14 of infection. (A) 10x magnification H&E staining of cecum mucosa in Day 14 infected mice. The lamina propia between the epithelium and muscularis mucosa has visible invasion of leukocytes, separating the epithelium and the muscularis mucosa. (B) 40x magnification of trichomonads via H&E staining. Within the highlighted region are the trichomonads. (C) 10x magnification H&E staining of cecum mucoa in Day 14 control mice. The lamina propia between the epithelium and muscularis mucosa has no separation.

In vivo efficacy of 5-NI compounds in animal infection models

The mice were monitored based off of fecal sampling on Day 1 and Day 2 post-inoculation to ensure that infection had occurred. Four days after the mice were infected, they were started on the dose regimen and given 6 doses over the course of three days. One day after the compound treatment, fecal samples were collected from the mice to determine whether there were detectable levels of trichomonads in the feces immediately following the complete treatment regimen. The mice used in the study were inducible Cre/loxP system models. They contained a region of their gene that was excisable under the influence of tamoxifen exposure; however, without tamoxifen the mice remained wildtype.

Four compounds in total were used to screen against the intestinal infection model developed (Figure 5), with their associated *in vitro* EC50 and pEC50 values shown (Table 1). These 4 compounds were selected as an average representation of the EC_{50} values of the 52 compounds that were more effective than Rz, when compared to the EC_{50} value of strain 340758+. Four of the 52 compounds have their in vitro values shown (Table 1) as well as the associated structures of these compounds (Figure 5). The 20 compounds with values also shown are a representative of activities of the compounds that were found to be more effective (Table 1)

Table 1. EC_{50} and pEC_{50} values of selected compounds from the 52 that were more efficacious than Rz.

	T. foetus 364287+		*T. foetus* 347058+		*T. foetus* 2002-96+++	
Compounds	**pEC_{50}**	**EC_{50} (μM)**	**pEC_{50}**	**EC_{50} (μM)**	**pEC_{50}**	**EC_{50} (μM)**
F-113	6.67 ± 0.13	0.21	4.78 ± 0.05	16.47	4.80 ± 0.03	15.73
E-245	6.38 ± 0.13	0.41	5.88 ± 0.13	1.33	5.74 ± 0.12	1.82
C-225	6.13 ± 0.05	0.75	5.68 ± 0.09	2.07	5.60 ± 0.15	2.51
B-156	6.09 ± 0.07	0.81	6.41 ± 0.11	0.39	6.33 ± 0.16	0.46
A-220	6.09 ± 0.13	0.82	6.32 ± 0.17	0.47	5.90 ± 0.12	1.27
B-135	6.05 ± 0.13	0.89	5.96 ± 0.07	1.10	5.90 ± 0.13	1.25
E-121	6.02 ± 0.06	0.95	5.53 ± 0.05	2.95	5.60 ± 0.04	2.51
F-123	6.00 ± 0.09	0.99	4.94 ± 0.14	11.48	4.70 ± 0.00	19.95
B-157	5.99 ± 0.12	1.04	5.23 ± 0.12	5.89	4.94 ± 0.10	11.48
C-231	5.96 ± 0.09	1.10	5.40 ± 0.07	3.95	5.37 ± 0.08	4.27
F-105	5.96 ± 0.07	1.10	5.15 ± 0.12	7.03	5.26 ± 0.08	5.54
B-131	5.95 ± 0.11	1.11	5.65 ± 0.07	2.24	5.75 ± 0.11	1.79
B-235	5.91 ± 0.09	1.22	5.38 ± 0.04	4.20	5.50 ± 0.10	3.14
C-112	5.90 ± 0.07	1.25	5.84 ± 0.12	1.43	5.57 ± 0.11	2.69
C-158	5.88 ± 0.08	1.33	6.14 ± 0.06	0.72	6.32 ± 0.11	0.48
F-207	5.77 ± 0.30	1.70	5.14 ± 0.08	7.19	4.99 ± 0.04	10.31
E-218	5.42 ± 0.13	3.80	5.01 ± 0.04	9.77	4.95 ± 0.04	11.31
E-216	5.40 ± 0.06	3.95	5.36 ± 0.05	4.40	5.28 ± 0.03	5.21
F-230	5.17 ± 0.16	6.71	4.76 ± 0.03	17.38	4.70 ± 0.00	19.95
Rz	5.65 ± 0.24	2.24	5.85 ± 0.10	1.42	5.40 ± 0.06	4.02

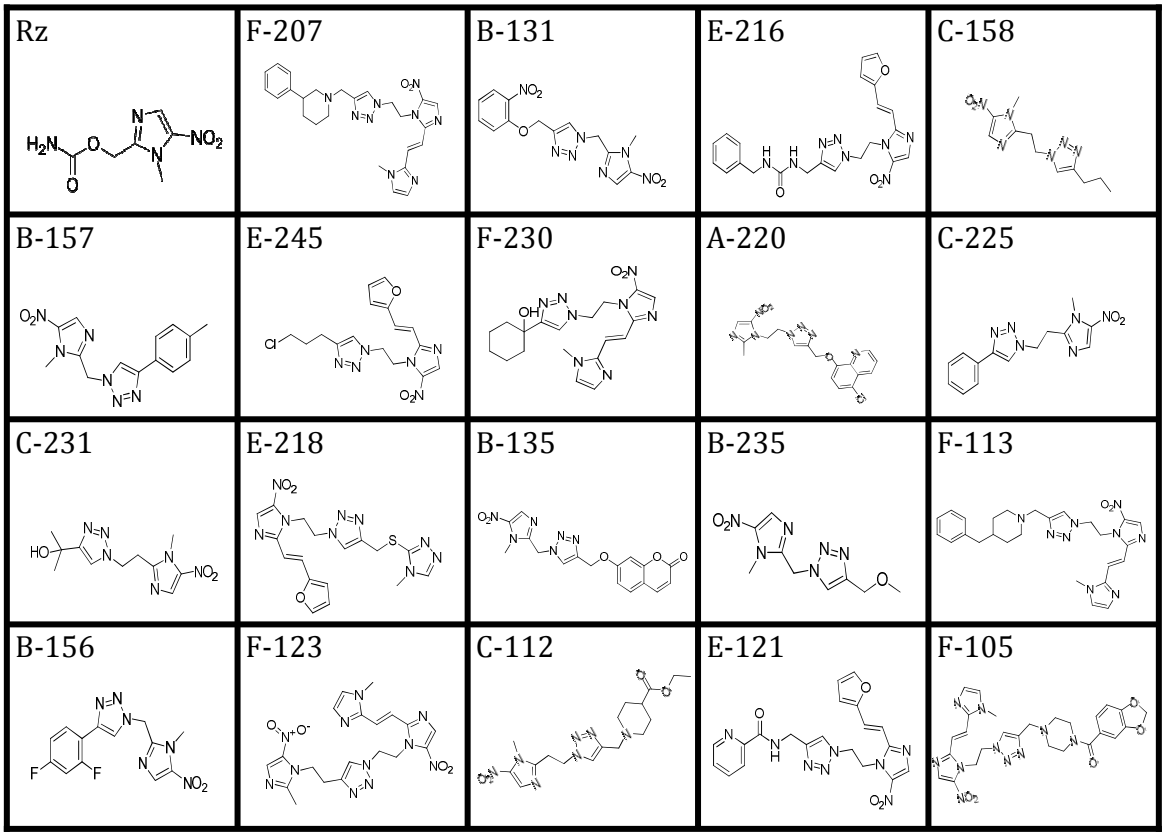


Figure 5. Structures of selected compounds.

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As expected from the intestinal infection model that was generated, the infection levels began to remain constant after Day 4. Every group, except for the control group, had a decrease in the active tritrichomonads found in the feces on Day 7, one day after the last dosage regimen. I expected that the tritrichomonad population to decrease, however there was not a significant decrease in the trichomonads counted in every experimental group except for compound E-218 (Figure 6). Comparing the population of *T. foetus* in each mouse prior to the treatment to the population in each mouse after the treatment was completed. On average, each group had a smaller population of tritrichomonads in the feces; however, there was no significant decrease in three experimental groups when compared to the control mice. Only mice given E-218 showed a significant decrease ($p<0.05$) in the trichomonad population when compared to control (Figure 6). Comparing each group's population prior to and after the treatment regimen, each experimental group had a population lower than the control. However, only group E-218 had a significant inhibition and reduction of the tritrichomonad population by 85%, when compared its Day 4 tritrichomonad fecal count (Figure 6). While group F-230 had both an inhibition and reduction of its tritrichomonad population by 8%, this was not a significant reduction from the population percentage increase observed in the control mice (Figure 6). Both groups E-216 and F-207 had an increase in the trichomonad population after the final treatment day, 29% and 74% respectively (Figure 6). Compared to the control group's 137% increase in tritrichomonads, compounds E-216 and F-207 showed an inhibition of the tritrichomonad colonization; however, this inhibition was not significantly different upon statistical analysis (Figure 6).

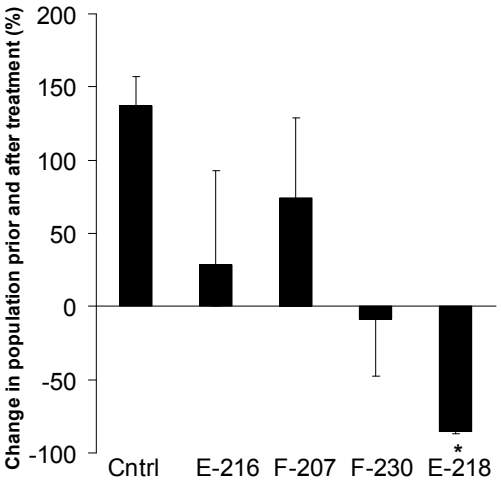

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Figure 6. Infection and treatment progression in intestinal 364287+ infection model. * ($p<0.05$)

Discussion

An effective way of studying new 5-NI compounds against feline *T. foetus* infections in small rodent intestinal infection models has not been previously reported. This study shows that careful screening of newly derived compounds and establishment a stable and constant infection in murine models allow for the screening of a new class of compounds that would be effective against *T. foetus* infections (Valdez *et al.* 2009). Comparing each of the values against the pEC_{50} value that was set for Rz for each strain of tritrichomonad allowed us to determine which compounds would be potentially more effective for further studies (Miyamoto *et al.* 2013). Establishing this process for screening a pathogen under both in vivo and in vitro circumstances sets a standard for approaching drug development.

Testing the compounds and selecting which compounds were more effective than Rz in vitro purely on EC_{50} values was the only reliable method for determining new possible compounds (Upcroft *et al.* 2006). When studied in retrospective cases, Rz was not been proven to be entirely effective, due to conflicting claims, relapse in infections, and inability to clear the pathogen entirely (Xenoulis *et al.* 2013). As a result, having a new set of compounds that has been proven to be effective under both in vitro and in vivo conditions sets the stage for further development of those compounds. By screening these compounds multiple times and against multiple *T. foetus* strains, the library of compounds quickly develops into a set of compounds that may contain structural similarities.

Currently there have been suggestions that *T. foetus* in bovine infections is of a completely different species than that of the species that infects the intestinal tract of

felines (Walden *et al.* 2013). The suggestion has been that the feline strain of *T. foetus* is in fact the strain *Tritrichomonas suis,* the pathogenic strain of *tritrichomonas* that infects the nasal cavity and gastrointestinal tract of porcine. Due to the similarities in infection pathways and morphology, changing the nomenclature of feline *T. foetus* to *T. suis* should be considered and further evaluated (Doi *et al.* 2012). Although the metabolic and cytotoxic pathways may be similar between bovine and feline *T. foetus*, their infection profiles are significantly different, which was elucidated when a group tried to mimic the effects of feline intestinal infections when infected with bovine derived *T. foetus* strains (Stockdale *et al.* 2008). The results showed that the infection intensity and profiles did not have a significant similarity between the two strains (Stockdale *et al.* 2008). However, further analysis and PCR data is required to quantify these distinctions before being fully accepted by veterinary clinicians (Gruffydd-Jones *et al.* 2013). This discrepancy between the two possible strains of tritrichomonads would explain the different compounds that are used to treat the infection. Therefore, more information is needed to elucidate the metabolic profiles of each species.

In this present study, a murine model for intestine feline *T. foetus* infections was generated in order to understand the response that tritrichomonad would have during in vivo studies. While initially the establishment of intestinal tritrichomonad murine infection was a promising start for possibly studying the pathogenicity of *T. foetus*, the lack of development of chronic large-bowel diarrhea prevented furthering this field of study (Yaeger and Gookin, 2005). This lack of a large diarrheal response could be due in part to the age of the mice, similarly to what is observed clinically in felines. The older the cat the more likely they become asymptomatic for *T. foetus* infection. While being

unable to study the pathogenicity of the tritrichomonads in this murine model, the constant high sustainable levels of tritrichomonad colonization allows for furthering the study of various compound's in vivo potentials. Shown in this study by the observation of the limited exposure experimental group was able to maintain significant levels of infections 4 weeks post challenge date, while the sustained antibiotic group never had a significant decrease in the levels of infection. However, the limited antibiotic exposure group models a system with less external influences which can have adverse effects while studying drug interaction with the parasite. The difficulty of decreasing the amount of external manipulations can be attributed to observation the murine host system seems to develop a response to the tritrichomonads and are able to clear them from their system. Thus there could be a drawback in studying drug effects on the parasite since the decrease in trichomonad levels could not necessarily be completely attributed to the effects of the compounds. As a result, in order to sustain a reliable infection level to study the in vivo effects of the 5-NI compounds, a parallel course of antibiotics were given to the mice. Therefore, the model satisfied the criterion which was required to test the new 5-NI compounds in vivo. However, the model did differ from the principle host organism based on the fact that the presence of chronic large scale diarrhea was not present in the murine models. Quantifying diarrheal response via fecal water mass was the main method of detecting the symptoms of diarrhea.

While this is an observable symptom found in domestic felines, the murine model did not have a significant difference between the antibiotic and tritrichomonad infected mice when compared to mice kept only on antibiotics (data not shown). Due to the fact that mice kept on antibiotics for a long period of time were able to develop diarrhea, the

development of increased water mass in response to the infection in the murine infection model could not be interpreted to be caused by the pathogenic parasite alone. Due to the fact that mice kept on antibiotics for a long duration of time will also develop diarrhea, therefore being unable to determine whether the diarrhea was caused by the pathogen or the antibiotic conditioning. Paralleled with the fact that primarily only kittens were able to develop a diarrheal response to *T. foetus* infections, while older cats did not, the ability to see that in our murine models would be difficult to determine since the life spans of mice and cats differ greatly (Gookin *et al.* 2004). This age specific symptom development could possibly explain the fact that there was no significant differences in mice that were treated with antibiotics versus mice also infected with tritrichomonas.

Although the use of antibiotics is an external manipulation of the host organism, this remains a viable method for establishing infection models for other pathogens such as *T. vaginalis* and *G. lambila* (Cobo *et al.* 2011) (Tejman-Yarden *et al.* 2011). The use of the antibiotics is crucial due to the fact that this particular species of *Tritrichomonas* is not the principal species that infects mice. As a result, antibiotics are required to remove the native flora and allow the trichomonads to establish an infective niche in the murine models. Being able to establish a murine intestinal *T. foetus* model allows for the relief of continuing testing methods on felines. Small rodent models are generated easily, highly controlled, well maintained and monitored, and require less space and handling. Although it was not observed that *T. foetus* caused inflammation in the large intestine mucosa of the small rodent models, *T. foetus* did cause inflammation in the cecal mucosa. While, these two organs are functionally similar, the principle host organism, domestic felines, anatomically do not contain a cecum, although the cecum is considered to be part of the

large intestine. The inflammation and invasion of the pathogen into the cecal mucosa in murine models may be due to the increase in volume from the small intestine as well as the decrease in intestinal motility allowing the pathogen to establish a strong presence in the cecum. Decreasing the motility of substances Similar to the feline intestinal tract, the junction between the small intestine and the large intestine is greatly increased in volume therefore slowing the velocity of the intestinal contents. Although this infection model does not mirror the diarrheal symptoms developed by cats when infected with *T. foetus*, this murine model, however, does maintain a long sustainable infection as well as contain a host inflammatory response to the *T. foetus* infection, which is similar to the observations seen in felines (Yaeger and Gookin, 2005). However, whether the murine host develops an innate response to the trichomonad infection is a parameter that requires further testing via ELISA and antibody tests. The antibodies could be IgA or IgG due to the nature of the host response and the method by which the immune system and the complimentary system responds to a pathogenic infection in the intestines.

Nitroimidazoles, including Rz, remain the mainstay of chemotherapeutic agents against feline trichomonaisis. Infecting mice with 364287+ strain of feline intestinal *T. foetus* to assess the infection model for chemotherapeutic potentials is a new field of research. The dose used in this study was enough to inhibit the growth of the *T. foetus*, however, the dose was not high enough to reduce the population of *T. foetus* in each group. More studies are required to determine the dosing or number of doses that is necessary to completely clear the infection. In our current in vivo study, the reduction of trichomonad population showed that the compounds were effective. Although at a concentration of 10 mg/ kg does not seem to be effective enough to clear the infection

from the host. Comparing the initial growth just prior to the initial dose and the final dose, representing these differences in percentages, was the method used to understand the effects of the compounds under in vivo conditions. More studies have to be done to understand the effects of each compound under in vivo conditions as well as the proper dosing amount and duration.

In conclusion, this study shows in vitro testing conditions necessary for screening a large library of compounds and developing an efficient manner for the further development of a new group of 5-NI derived drugs. Paralleled with the development of a suitable and measurable method for an intestinal *T. foetus* murine infection model helped further the development of the new compounds. This infection model allows for the testing of the selected compounds in vivo potentials, which allows for the development of a few compounds for further study. The in vitro and in vivo studies of feline tritruchomonasis in murine models and their responses to newly derived 5-NIcompounds may become a useful tool for selecting and furthering the study of therapeutic drugs.

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Supplemental Data

Table S1. D1 and 364287+ strain responses against the 5-NI compound library EC50 and pEC50 values with standard errors shown.

		T.foetus D1s		*T.foetus* 364287+	
Compounds	**EC50 (uM)**	**pEC50**		**EC50 (uM)**	**pEC50**
Mz	0.26	6.58 ± 0.00		2.07	5.68 ± 0.00
Rz	0.78	6.11 ± 0.18		2.24	5.65 ± 0.24
A-101	0.61	6.22 ± 0.06		5.45	5.26 ± 0.10
A-102	0.22	6.66 ± 0.09		3.92	5.41 ± 0.14
A-103	0.02	7.66 ± 0.23		19.95	4.70 ± 0.00
A-104	0.19	6.71 ± 0.09		19.95	4.70 ± 0.00
A-105	0.10	7.01 ± 0.10		3.89	5.41 ± 0.24
A-106	0.29	6.53 ± 0.07		6.17	5.21 ± 0.15
A-107	0.30	6.52 ± 0.08		7.53	5.12 ± 0.24
A-108	0.18	6.75 ± 0.18		4.57	5.34 ± 0.30
A-109	0.30	6.52 ± 0.09		9.26	5.03 ± 0.10
A-110	0.38	6.42 ± 0.12		14.68	4.83 ± 0.08
A-111	0.59	6.23 ± 0.10		11.93	4.92 ± 0.11
A-112	0.10	7.00 ± 0.16		2.57	5.59 ± 0.10
A-113	0.13	6.90 ± 0.12		8.58	5.07 ± 0.10
A-114	3.07	5.51 ± 0.06		19.95	4.70 ± 0.00
A-115	4.75	5.32 ± 0.03		19.95	4.70 ± 0.00
A-116	4.68	5.33 ± 0.05		16.60	4.78 ± 0.05
A-117	0.06	7.22 ± 0.91		2.29	5.64 ± 0.31
A-118	0.22	6.67 ± 0.17		11.39	4.94 ± 0.12
A-119	0.45	6.35 ± 0.09		16.22	4.79 ± 0.05
A-120	0.91	6.04 ± 0.00		19.95	4.70 ± 0.00
A-121	0.19	6.71 ± 0.00		3.89	5.41 ± 0.16
A-122	0.34	6.47 ± 0.00		14.34	4.84 ± 0.08
A-123	2.29	5.64 ± 0.00		19.95	4.70 ± 0.00
A-124	2.51	5.60 ± 0.00		19.95	4.70 ± 0.00
A-125	0.43	6.36 ± 0.04		19.95	4.70 ± 0.00
A-126	0.26	6.59 ± 0.12		18.48	4.73 ± 0.02
A-127	0.41	6.39 ± 0.04		9.70	5.01 ± 0.10
A-128	1.47	5.83 ± 0.11		19.95	4.70 ± 0.00
A-129	0.91	6.04 ± 0.07		19.95	4.70 ± 0.00
A-130	0.19	6.73 ± 0.22		19.95	4.70 ± 0.00
A-131	1.30	5.89 ± 0.05		7.13	5.15 ± 0.26
A-132	N/A	N/A ± N/A		4.68	5.33 ± 0.36
A-133	0.14	6.86 ± 0.03		1.82	5.74 ± 0.10
A-134	0.19	6.73 ± 0.06		2.09	5.68 ± 0.03
A-135	0.49	6.31 ± 0.06		9.33	5.03 ± 0.13
A-136	1.30	5.89 ± 0.08		6.17	5.21 ± 0.16
A-137	4.03	5.40 ± 0.05		16.60	4.78 ± 0.05
A-138	0.23	6.64 ± 0.11		6.51	5.19 ± 0.11
A-139	0.10	6.99 ± 0.06		1.75	5.76 ± 0.12
A-140	0.19	6.73 ± 0.13		4.79	5.32 ± 0.18
A-141	0.45	6.34 ± 0.08		13.18	4.88 ± 0.05

A-142	4.14	5.38 ± 0.02	19.95	4.70 ± 0.00
A-143	0.35	6.45 ± 0.08	3.07	5.51 ± 0.01
A-144	2.59	5.59 ± 0.06	19.95	4.70 ± 0.00
A-145	9.48	5.02 ± 0.06	19.95	4.70 ± 0.00
A-146	3.63	5.44 ± 0.04	19.95	4.70 ± 0.00
A-147	0.46	6.33 ± 0.07	6.21	5.21 ± 0.23
A-148	0.02	7.79 ± 0.08	1.81	5.74 ± 0.06
A-149	0.34	6.47 ± 0.09	4.57	5.34 ± 0.04
A-150	1.14	5.94 ± 0.04	17.11	4.77 ± 0.03
A-151	0.52	6.28 ± 0.24	19.95	4.70 ± 0.00
A-152	5.37	5.27 ± 0.04	19.95	4.70 ± 0.00
A-153	1.15	5.94 ± 0.05	19.95	4.70 ± 0.00
A-154	0.15	6.83 ± 0.10	2.75	5.56 ± 0.29
A-155	0.25	6.61 ± 0.07	7.59	5.12 ± 0.03
A-156	0.18	6.75 ± 0.10	6.56	5.18 ± 0.09
A-157	0.33	6.48 ± 0.07	7.76	5.11 ± 0.05
A-158	0.08	7.10 ± 0.12	0.99	6.00 ± 0.28
A-159	0.77	6.11 ± 0.08	13.18	4.88 ± 0.04
A-160	0.65	6.19 ± 0.08	10.59	4.98 ± 0.01
A-161	1.25	5.90 ± 0.07	19.05	4.72 ± 0.01
A-162	0.34	6.46 ± 0.07	8.45	5.07 ± 0.16
A-163	0.11	6.94 ± 0.05	5.99	5.22 ± 0.10
A-201	0.86	6.07 ± 0.05	15.73	4.80 ± 0.06
A-202	0.20	6.70 ± 0.10	4.61	5.34 ± 0.11
A-203	0.04	7.42 ± 0.13	3.41	5.47 ± 0.10
A-204	0.02	7.69 ± 0.11	1.92	5.72 ± 0.07
A-205	0.28	6.56 ± 0.12	5.80	5.24 ± 0.12
A-206	0.76	6.12 ± 0.11	19.95	4.70 ± 0.00
A-207	0.22	6.66 ± 0.14	6.51	5.19 ± 0.12
A-208	0.11	6.96 ± 0.15	11.05	4.96 ± 0.08
A-209	0.05	7.33 ± 0.10	4.94	5.31 ± 0.15
A-210	0.14	6.85 ± 0.23	1.23	5.91 ± 0.12
A-211	0.52	6.29 ± 0.17	14.91	4.83 ± 0.05
A-212	0.34	6.47 ± 0.09	5.25	5.28 ± 0.13
A-213	0.41	6.39 ± 0.09	10.80	4.97 ± 0.07
A-214	0.16	6.80 ± 0.00	4.23	5.37 ± 0.31
A-215	0.79	6.10 ± 0.00	14.91	4.83 ± 0.04
A-216	0.78	6.11 ± 0.07	19.95	4.70 ± 0.00
A-217	0.20	6.70 ± 0.07	12.59	4.90 ± 0.12
A-218	N/A	N/A ± N/A	19.95	4.70 ± 0.00
A-219	0.14	6.86 ± 0.06	0.82	6.09 ± 0.13
A-220	0.03	7.48 ± 0.06	0.54	6.27 ± 0.17
A-221	16.60	4.78 ± 0.00	19.95	4.70 ± 0.00
A-222	0.07	7.18 ± 0.08	1.29	5.89 ± 0.08
A-223	0.52	6.28 ± 0.07	6.56	5.18 ± 0.12
A-224	0.09	7.03 ± 0.08	3.98	5.40 ± 0.21
A-225	0.21	6.67 ± 0.07	3.92	5.41 ± 0.16
A-226	0.09	7.03 ± 0.05	0.97	6.01 ± 0.13
A-227	0.16	6.79 ± 0.07	2.95	5.53 ± 0.24
A-228	0.21	6.67 ± 0.06	3.41	5.47 ± 0.11

A-229	4.14	5.38 ± 0.08	19.95	4.70 ± 0.00
A-230	0.23	6.64 ± 0.13	7.47	5.13 ± 0.13
A-231	0.27	6.57 ± 0.11	8.71	5.06 ± 0.13
A-232	0.75	6.13 ± 0.09	12.69	4.90 ± 0.06
A-233	0.33	6.49 ± 0.05	2.48	5.61 ± 0.17
A-234	1.31	5.88 ± 0.07	18.20	4.74 ± 0.01
A-235	2.51	5.60 ± 0.04	19.95	4.70 ± 0.00
A-236	0.27	6.57 ± 0.13	12.02	4.92 ± 0.07
A-237	0.22	6.66 ± 0.15	13.80	4.86 ± 0.09
A-238	1.05	5.98 ± 0.08	16.47	4.78 ± 0.03
A-239	0.72	6.14 ± 0.13	10.80	4.97 ± 0.09
A-240	0.07	7.14 ± 0.10	1.89	5.72 ± 0.06
A-241	1.05	5.98 ± 0.07	13.91	4.86 ± 0.05
A-242	0.19	6.71 ± 0.06	15.25	4.82 ± 0.05
A-243	0.09	7.03 ± 0.11	3.41	5.47 ± 0.08
A-244	1.05	5.98 ± 0.07	15.25	4.82 ± 0.04
A-245	0.11	6.95 ± 0.06	1.98	5.70 ± 0.03
A-246	19.95	4.70 ± 0.00	19.95	4.70 ± 0.00
A-247	0.47	6.33 ± 0.04	10.35	4.99 ± 0.14
B-101	0.17	6.77 ± 0.04	3.66	5.44 ± 0.22
B-102	0.33	6.48 ± 0.03	9.61	5.02 ± 0.10
B-103	0.04	7.43 ± 0.09	1.27	5.90 ± 0.06
B-104	0.87	6.06 ± 0.04	18.30	4.74 ± 0.02
B-105	0.97	6.01 ± 0.05	19.95	4.70 ± 0.00
B-106	1.19	5.92 ± 0.06	18.09	4.74 ± 0.02
B-107	0.29	6.53 ± 0.02	11.57	4.94 ± 0.14
B-108	0.30	6.52 ± 0.27	15.49	4.81 ± 0.06
B-109	0.97	6.01 ± 0.07	17.18	4.77 ± 0.03
B-110	0.17	6.76 ± 0.16	9.23	5.04 ± 0.06
B-111	0.21	6.68 ± 0.08	7.82	5.11 ± 0.12
B-112	0.63	6.20 ± 0.02	15.40	4.81 ± 0.06
B-113	0.08	7.12 ± 0.14	1.46	5.84 ± 0.10
B-114	5.67	5.25 ± 0.03	19.95	4.70 ± 0.00
B-115	4.71	5.33 ± 0.03	19.95	4.70 ± 0.00
B-116	2.19	5.66 ± 0.06	19.16	4.72 ± 0.01
B-117	0.01	7.85 ± 0.07	3.55	5.45 ± 0.17
B-118	0.89	6.05 ± 0.02	9.44	5.03 ± 0.08
B-119	1.40	5.85 ± 0.09	17.08	4.77 ± 0.03
B-120	1.21	5.92 ± 0.17	19.95	4.70 ± 0.00
B-121	3.11	5.51 ± 0.06	19.95	4.70 ± 0.00
B-122	2.69	5.57 ± 0.08	19.95	4.70 ± 0.00
B-123	0.19	6.72 ± 0.13	1.91	5.72 ± 0.04
B-124	0.11	6.96 ± 0.08	2.47	5.61 ± 0.04
B-125	11.22	4.95 ± 0.02	19.95	4.70 ± 0.00
B-126	0.60	6.22 ± 0.13	7.46	5.13 ± 0.17
B-127	0.01	7.91 ± 0.07	0.60	6.22 ± 0.06
B-128	0.01	8.00 ± 0.11	1.05	5.98 ± 0.12
B-129	0.02	7.69 ± 0.12	0.68	6.17 ± 0.06
B-130	0.01	8.01 ± 0.03	3.41	5.47 ± 0.11
B-131	0.02	7.66 ± 0.02	1.11	5.95 ± 0.11

B-132	0.38	6.42 ± 0.04	7.24	5.14 ± 0.08
B-133	0.14	6.86 ± 0.06	3.47	5.46 ± 0.07
B-134	0.03	7.49 ± 0.06	0.36	6.45 ± 0.06
B-135	0.04	7.39 ± 0.06	0.89	6.05 ± 0.13
B-136	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-137	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-138	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-139	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-140	0.06	7.21 ± 0.04	0.65	6.19 ± 0.10
B-141	0.05	7.26 ± 0.06	1.20	5.92 ± 0.04
B-142	1.33	5.88 ± 0.04	7.36	5.13 ± 0.03
B-143	0.09	7.02 ± 0.08	2.71	5.57 ± 0.03
B-144	0.65	6.19 ± 0.01	7.70	5.11 ± 0.06
B-145	0.11	6.95 ± 0.19	2.06	5.69 ± 0.29
B-146	6.21	5.21 ± 0.01	19.95	4.70 ± 0.00
B-147	2.12	5.67 ± 0.03	19.95	4.70 ± 0.00
B-148	0.01	7.90 ± 0.10	1.00	6.00 ± 0.13
B-149	0.44	6.35 ± 0.05	9.48	5.02 ± 0.12
B-150	2.19	5.66 ± 0.06	5.98	5.22 ± 0.17
B-151	1.34	5.87 ± 0.04	16.98	4.77 ± 0.04
B-152	0.01	8.20 ± 0.08	0.30	6.52 ± 0.02
B-153	3.04	5.52 ± 0.04	6.31	5.20 ± 0.29
B-154	6.46	5.19 ± 0.06	16.09	4.79 ± 0.05
B-155	0.01	7.98 ± 0.10	2.43	5.62 ± 0.05
B-156	0.01	7.85 ± 0.04	0.81	6.09 ± 0.07
B-157	0.04	7.43 ± 0.13	1.04	5.99 ± 0.12
B-158	0.03	7.46 ± 0.05	0.83	6.08 ± 0.04
B-159	0.42	6.37 ± 0.09	8.81	5.06 ± 0.14
B-160	1.67	5.78 ± 0.12	15.67	4.81 ± 0.07
B-161	0.05	7.28 ± 0.06	0.74	6.13 ± 0.11
B-162	0.02	7.66 ± 0.06	0.58	6.24 ± 0.05
B-163	0.01	7.91 ± 0.07	0.62	6.21 ± 0.09
B-201	1.09	5.96 ± 0.08	19.95	4.70 ± 0.00
B-202	0.09	7.04 ± 0.11	9.83	5.01 ± 0.09
B-203	0.11	6.98 ± 0.05	4.37	5.36 ± 0.13
B-204	0.03	7.59 ± 0.07	3.47	5.46 ± 0.22
B-205	3.16	5.50 ± 0.08	19.95	4.70 ± 0.00
B-206	0.32	6.49 ± 0.07	12.66	4.90 ± 0.06
B-207	0.04	7.43 ± 0.25	0.38	6.42 ± 0.16
B-208	1.70	5.77 ± 0.07	19.95	4.70 ± 0.00
B-209	0.02	7.80 ± 0.10	6.84	5.17 ± 0.10
B-210	0.86	6.07 ± 0.15	13.65	4.87 ± 0.06
B-211	2.29	5.64 ± 0.00	19.95	4.70 ± 0.00
B-212	0.49	6.31 ± 0.11	14.54	4.84 ± 0.07
B-213	0.28	6.55 ± 0.09	4.90	5.31 ± 0.12
B-214	0.61	6.22 ± 0.16	7.51	5.12 ± 0.07
B-215	3.77	5.42 ± 0.13	11.89	4.93 ± 0.11
B-216	1.25	5.90 ± 0.14	19.95	4.70 ± 0.00
B-217	0.16	6.79 ± 0.12	3.57	5.45 ± 0.05
B-218	2.07	5.68 ± 0.01	18.20	4.74 ± 0.03

B-219	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-220	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-221	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-222	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-223	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-224	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-225	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-226	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-227	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-228	N/A	N/A ± N/A	19.95	4.70 ± 0.00
B-229	0.31	6.51 ± 0.08	4.11	5.39 ± 0.07
B-230	1.36	5.87 ± 0.12	10.00	5.00 ± 0.08
B-231	1.51	5.82 ± 0.06	9.62	5.02 ± 0.07
B-232	0.23	6.64 ± 0.06	5.37	5.27 ± 0.08
B-233	0.12	6.91 ± 0.05	0.98	6.01 ± 0.04
B-234	4.20	5.38 ± 0.03	17.99	4.75 ± 0.03
B-235	0.09	7.03 ± 0.06	1.22	5.91 ± 0.09
B-236	0.03	7.48 ± 0.11	1.29	5.89 ± 0.10
B-237	0.02	7.63 ± 0.07	1.25	5.90 ± 0.07
B-238	2.65	5.58 ± 0.04	19.95	4.70 ± 0.00
B-239	0.46	6.34 ± 0.11	9.12	5.04 ± 0.06
B-240	0.30	6.52 ± 0.10	5.67	5.25 ± 0.08
B-241	0.01	8.04 ± 0.07	1.82	5.74 ± 0.03
B-242	0.01	8.06 ± 0.10	1.62	5.79 ± 0.11
B-243	0.02	7.82 ± 0.13	0.93	6.03 ± 0.08
B-244	0.00	8.43 ± 0.03	0.10	6.99 ± 0.14
B-245	0.03	7.50 ± 0.03	1.12	5.95 ± 0.09
B-246	0.02	7.75 ± 0.05	0.44	6.36 ± 0.15
B-247	0.03	7.53 ± 0.04	0.34	6.47 ± 0.10
C-101	0.03	7.52 ± 0.05	1.07	5.97 ± 0.07
C-102	0.03	7.51 ± 0.13	1.11	5.95 ± 0.04
C-103	0.02	7.71 ± 0.07	1.85	5.73 ± 0.07
C-104	0.12	6.92 ± 0.16	4.87	5.31 ± 0.10
C-105	0.43	6.36 ± 0.05	7.99	5.10 ± 0.06
C-106	0.18	6.76 ± 0.16	5.16	5.29 ± 0.10
C-107	0.06	7.21 ± 0.15	1.87	5.73 ± 0.04
C-108	0.19	6.72 ± 0.08	6.35	5.20 ± 0.05
C-109	0.23	6.63 ± 0.08	7.24	5.14 ± 0.02
C-110	0.10	7.01 ± 0.10	5.28	5.28 ± 0.07
C-111	0.03	7.59 ± 0.12	1.40	5.86 ± 0.05
C-112	0.02	7.64 ± 0.12	1.25	5.90 ± 0.07
C-113	0.05	7.31 ± 0.01	1.50	5.82 ± 0.03
C-114	0.12	6.93 ± 0.13	5.62	5.25 ± 0.05
C-115	0.14	6.85 ± 0.11	6.76	5.17 ± 0.06
C-116	0.05	7.34 ± 0.21	1.21	5.92 ± 0.05
C-117	0.12	6.91 ± 0.09	10.23	4.99 ± 0.10
C-118	0.01	7.90 ± 0.09	4.05	5.39 ± 0.08
C-119	0.21	6.68 ± 0.16	4.87	5.31 ± 0.08
C-120	0.23	6.63 ± 0.09	3.76	5.43 ± 0.07
C-121	0.08	7.09 ± 0.06	1.80	5.75 ± 0.06

C-122	0.16	6.81 ± 0.32	6.65	5.18 ± 0.07
C-123	0.09	7.02 ± 0.13	1.86	5.73 ± 0.06
C-124	0.13	6.89 ± 0.21	5.62	5.25 ± 0.05
C-125	0.28	6.56 ± 0.12	5.07	5.30 ± 0.12
C-126	0.08	7.10 ± 0.10	10.00	5.00 ± 0.12
C-127	0.18	6.73 ± 0.09	17.38	4.76 ± 0.03
C-128	0.32	6.49 ± 0.08	19.95	4.70 ± 0.00
C-129	0.23	6.64 ± 0.09	19.95	4.70 ± 0.00
C-130	0.01	8.14 ± 0.01	2.65	5.58 ± 0.26
C-131	0.01	7.89 ± 0.03	4.37	5.36 ± 0.16
C-132	0.63	6.20 ± 0.08	18.41	4.74 ± 0.02
C-133	0.06	7.25 ± 0.08	4.90	5.31 ± 0.06
C-134	0.03	7.51 ± 0.09	2.66	5.58 ± 0.22
C-135	0.11	6.95 ± 0.05	15.14	4.82 ± 0.04
C-136	0.11	6.97 ± 0.05	3.44	5.46 ± 0.02
C-137	0.20	6.70 ± 0.07	6.03	5.22 ± 0.20
C-138	0.02	7.75 ± 0.01	0.21	6.68 ± 0.13
C-139	0.01	7.92 ± 0.12	0.16	6.79 ± 0.22
C-140	0.09	7.06 ± 0.04	1.69	5.77 ± 0.12
C-141	0.01	8.07 ± 0.08	0.89	6.05 ± 0.12
C-142	0.45	6.35 ± 0.09	9.33	5.03 ± 0.08
C-143	0.04	7.37 ± 0.09	0.91	6.04 ± 0.06
C-144	0.27	6.57 ± 0.11	2.38	5.62 ± 0.24
C-145	0.04	7.42 ± 0.04	2.20	5.66 ± 0.08
C-146	0.47	6.33 ± 0.19	12.16	4.92 ± 0.07
C-147	0.02	7.69 ± 0.11	4.60	5.34 ± 0.10
C-148	0.01	8.23 ± 0.06	1.69	5.77 ± 0.18
C-149	0.09	7.02 ± 0.08	4.07	5.39 ± 0.07
C-150	0.26	6.58 ± 0.07	10.96	4.96 ± 0.05
C-151	0.16	6.80 ± 0.17	4.90	5.31 ± 0.12
C-152	0.05	7.30 ± 0.05	5.16	5.29 ± 0.06
C-153	0.10	6.99 ± 0.04	5.50	5.26 ± 0.10
C-154	0.40	6.39 ± 0.10	6.38	5.20 ± 0.12
C-155	0.03	7.49 ± 0.13	2.62	5.58 ± 0.06
C-156	0.06	7.20 ± 0.09	4.64	5.33 ± 0.12
C-157	0.16	6.81 ± 0.14	8.27	5.08 ± 0.05
C-158	0.04	7.41 ± 0.08	1.33	5.88 ± 0.08
C-159	0.28	6.55 ± 0.11	6.65	5.18 ± 0.07
C-160	0.19	6.72 ± 0.04	4.68	5.33 ± 0.08
C-161	0.06	7.21 ± 0.05	1.31	5.88 ± 0.11
C-162	0.12	6.91 ± 0.06	7.67	5.12 ± 0.06
C-163	0.03	7.48 ± 0.08	2.36	5.63 ± 0.04
C-201	0.30	6.52 ± 0.08	9.66	5.02 ± 0.10
C-202	0.02	7.72 ± 0.10	0.56	6.26 ± 0.12
C-203	0.02	7.63 ± 0.04	0.50	6.30 ± 0.12
C-204	0.01	7.87 ± 0.05	0.44	6.36 ± 0.07
C-205	0.07	7.14 ± 0.06	6.66	5.18 ± 0.04
C-206	0.21	6.68 ± 0.09	6.42	5.19 ± 0.07
C-207	0.03	7.49 ± 0.05	1.07	5.97 ± 0.03
C-208	0.14	6.86 ± 0.10	5.43	5.27 ± 0.06

C-209	0.01	8.07 ± 0.07	2.74	5.56 ± 0.21
C-210	0.06	7.25 ± 0.13	2.32	5.64 ± 0.06
C-211	0.14	6.85 ± 0.07	4.44	5.35 ± 0.07
C-212	0.13	6.89 ± 0.10	3.51	5.46 ± 0.07
C-213	0.21	6.68 ± 0.03	3.11	5.51 ± 0.08
C-214	0.12	6.93 ± 0.14	2.45	5.61 ± 0.04
C-215	0.17	6.78 ± 0.24	5.22	5.28 ± 0.07
C-216	0.25	6.60 ± 0.04	8.08	5.09 ± 0.06
C-217	0.11	6.94 ± 0.18	4.98	5.30 ± 0.06
C-218	0.52	6.28 ± 0.05	19.95	4.70 ± 0.00
C-219	0.02	7.64 ± 0.06	1.40	5.85 ± 0.09
C-220	0.01	7.87 ± 0.06	7.70	5.11 ± 0.24
C-221	0.33	6.48 ± 0.01	15.25	4.82 ± 0.04
C-222	0.02	7.79 ± 0.15	1.01	6.00 ± 0.24
C-223	0.08	7.08 ± 0.10	6.46	5.19 ± 0.12
C-224	0.03	7.52 ± 0.06	0.58	6.24 ± 0.03
C-225	0.02	7.66 ± 0.06	0.75	6.13 ± 0.05
C-226	0.01	7.86 ± 0.07	0.36	6.45 ± 0.07
C-227	0.01	7.93 ± 0.06	0.34	6.47 ± 0.04
C-228	0.01	7.95 ± 0.04	0.45	6.35 ± 0.11
C-229	0.04	7.40 ± 0.12	1.64	5.79 ± 0.07
C-230	0.10	7.02 ± 0.06	1.62	5.79 ± 0.13
C-231	0.10	7.00 ± 0.02	1.10	5.96 ± 0.09
C-232	0.39	6.41 ± 0.05	4.90	5.31 ± 0.02
C-233	0.04	7.45 ± 0.03	1.28	5.89 ± 0.05
C-234	0.26	6.59 ± 0.07	5.34	5.27 ± 0.06
C-235	0.09	7.03 ± 0.06	2.03	5.69 ± 0.04
C-236	0.18	6.75 ± 0.05	15.40	4.81 ± 0.03
C-237	0.03	7.57 ± 0.13	2.85	5.55 ± 0.08
C-238	0.27	6.56 ± 0.11	4.81	5.32 ± 0.08
C-239	0.37	6.43 ± 0.04	9.07	5.04 ± 0.06
C-240	0.09	7.06 ± 0.10	2.18	5.66 ± 0.18
C-241	0.03	7.52 ± 0.10	1.11	5.96 ± 0.06
C-242	0.03	7.49 ± 0.09	6.35	5.20 ± 0.08
C-243	0.01	8.13 ± 0.07	1.01	6.00 ± 0.07
C-244	0.01	8.14 ± 0.07	1.73	5.76 ± 0.08
C-245	0.03	7.60 ± 0.04	1.15	5.94 ± 0.04
C-246	0.05	7.33 ± 0.22	2.51	5.60 ± 0.15
C-247	0.05	7.28 ± 0.05	1.65	5.78 ± 0.04
D-101	14.96	4.83 ± 0.06	16.22	4.79 ± 0.05
D-102	15.49	4.81 ± 0.00	19.35	4.71 ± 0.01
D-103	8.71	5.06 ± 0.00	12.21	4.91 ± 0.06
D-104	N/A	N/A ± N/A	19.95	4.70 ± 0.00
D-105	N/A	N/A ± N/A	14.45	4.84 ± 0.08
D-106	12.30	4.91 ± 0.00	17.11	4.77 ± 0.04
D-107	12.02	4.92 ± 0.07	11.48	4.94 ± 0.12
D-108	N/A	N/A ± N/A	18.76	4.73 ± 0.02
D-109	N/A	N/A ± N/A	17.78	4.75 ± 0.03
D-110	11.48	4.94 ± 0.00	15.97	4.80 ± 0.06
D-111	14.13	4.85 ± 0.00	16.85	4.77 ± 0.04

D-112	12.02	4.92 ± 0.00	12.40	4.91 ± 0.07
D-113	12.30	4.91 ± 0.00	16.47	4.78 ± 0.05
D-114	N/A	N/A ± N/A	19.95	4.70 ± 0.00
D-115	15.49	4.81 ± 0.06	18.20	4.74 ± 0.02
D-116	N/A	N/A ± N/A	19.95	4.70 ± 0.00
D-117	N/A	N/A ± N/A	19.95	4.70 ± 0.00
D-118	7.94	5.10 ± 0.10	13.39	4.87 ± 0.10
D-119	N/A	N/A ± N/A	19.95	4.70 ± 0.00
D-120	N/A	N/A ± N/A	17.11	4.77 ± 0.04
D-121	N/A	N/A ± N/A	15.25	4.82 ± 0.07
D-122	N/A	N/A ± N/A	17.65	4.75 ± 0.03
D-123	4.32	5.37 ± 0.20	5.45	5.26 ± 0.11
D-124	7.59	5.12 ± 0.13	17.11	4.77 ± 0.04
D-125	N/A	N/A ± N/A	18.76	4.73 ± 0.02
D-126	N/A	N/A ± N/A	17.78	4.75 ± 0.03
D-127	14.96	4.83 ± 0.03	10.72	4.97 ± 0.16
D-128	11.35	4.95 ± 0.01	16.47	4.78 ± 0.05
D-129	12.74	4.90 ± 0.00	17.38	4.76 ± 0.03
D-130	6.03	5.22 ± 0.13	16.60	4.78 ± 0.05
D-131	N/A	N/A ± N/A	14.68	4.83 ± 0.04
D-132	N/A	N/A ± N/A	18.34	4.74 ± 0.02
D-133	N/A	N/A ± N/A	10.84	4.97 ± 0.19
D-134	5.50	5.26 ± 0.14	8.51	5.07 ± 0.08
D-135	13.80	4.86 ± 0.01	12.49	4.90 ± 0.12
D-136	12.45	4.91 ± 0.05	12.45	4.91 ± 0.14
D-137	14.29	4.85 ± 0.08	9.12	5.04 ± 0.20
D-138	9.12	5.04 ± 0.07	6.26	5.20 ± 0.12
D-139	8.41	5.08 ± 0.13	5.67	5.25 ± 0.11
D-140	8.13	5.09 ± 0.16	19.95	4.70 ± 0.00
D-201	N/A	N/A ± N/A	19.95	4.70 ± 0.00
D-202	13.03	4.89 ± 0.03	15.85	4.80 ± 0.07
D-203	12.74	4.90 ± 0.01	17.78	4.75 ± 0.03
D-204	9.02	5.05 ± 0.14	17.78	4.75 ± 0.03
D-205	N/A	N/A ± N/A	16.47	4.78 ± 0.05
D-206	N/A	N/A ± N/A	17.38	4.76 ± 0.03
D-207	14.13	4.85 ± 0.04	14.57	4.84 ± 0.08
D-208	10.72	4.97 ± 0.11	16.34	4.79 ± 0.05
D-209	4.68	5.33 ± 0.00	16.22	4.79 ± 0.05
D-210	7.76	5.11 ± 0.18	17.51	4.76 ± 0.03
D-211	N/A	N/A ± N/A	19.95	4.70 ± 0.00
D-212	16.98	4.77 ± 0.00	19.95	4.70 ± 0.00
D-213	13.18	4.88 ± 0.04	14.79	4.83 ± 0.08
D-215	8.61	5.07 ± 0.04	15.02	4.82 ± 0.07
D-216	N/A	N/A ± N/A	17.25	4.76 ± 0.04
D-217	N/A	N/A ± N/A	19.95	4.70 ± 0.00
D-218	10.59	4.98 ± 0.05	16.34	4.79 ± 0.05
D-219	11.61	4.94 ± 0.10	7.19	5.14 ± 0.12
D-220	N/A	N/A ± N/A	15.97	4.80 ± 0.06
D-221	N/A	N/A ± N/A	16.85	4.77 ± 0.04
D-222	11.89	4.93 ± 0.02	1.98	5.70 ± 0.07

D-223	N/A	N/A ± N/A	15.31	4.82 ± 0.08
D-224	11.75	4.93 ± 0.09	9.77	5.01 ± 0.22
D-225	6.53	5.19 ± 0.14	10.00	5.00 ± 0.04
D-226	7.08	5.15 ± 0.17	6.10	5.22 ± 0.10
D-227	4.73	5.33 ± 0.12	3.36	5.47 ± 0.16
D-228	6.92	5.16 ± 0.20	7.88	5.10 ± 0.11
D-229	9.02	5.05 ± 0.10	13.80	4.86 ± 0.11
D-230	13.80	4.86 ± 0.00	19.65	4.71 ± 0.00
D-231	N/A	N/A ± N/A	19.95	4.70 ± 0.00
D-232	N/A	N/A ± N/A	16.98	4.77 ± 0.04
D-233	16.03	4.80 ± 0.06	13.34	4.88 ± 0.12
D-234	N/A	N/A ± N/A	16.47	4.78 ± 0.05
D-235	7.00	5.16 ± 0.10	5.96	5.23 ± 0.20
E-101	0.10	6.99 ± 0.06	1.66	5.78 ± 0.07
E-102	0.07	7.16 ± 0.04	4.32	5.37 ± 0.05
E-103	0.01	8.00 ± 0.18	4.61	5.34 ± 0.10
E-104	0.04	7.35 ± 0.21	1.68	5.78 ± 0.76
E-105	0.03	7.55 ± 0.30	4.79	5.32 ± 0.07
E-106	0.19	6.72 ± 0.12	15.25	4.82 ± 0.07
E-107	0.02	7.75 ± 0.06	7.41	5.13 ± 0.16
E-108	0.02	7.82 ± 0.08	2.31	5.64 ± 0.11
E-109	0.10	7.01 ± 0.03	9.19	5.04 ± 0.10
E-110	0.03	7.50 ± 0.17	3.26	5.49 ± 0.26
E-111	0.81	6.09 ± 0.03	13.91	4.86 ± 0.05
E-112	0.07	7.17 ± 0.02	3.04	5.52 ± 0.08
E-113	0.04	7.35 ± 0.02	0.73	6.14 ± 0.06
E-114	0.26	6.59 ± 0.02	3.24	5.49 ± 0.03
E-115	0.09	7.05 ± 0.04	2.17	5.66 ± 0.07
E-116	0.09	7.05 ± 0.13	3.77	5.42 ± 0.11
E-117	2.34	5.63 ± 0.06	19.95	4.70 ± 0.00
E-118	0.10	7.01 ± 0.11	17.51	4.76 ± 0.03
E-119	0.08	7.09 ± 0.03	9.19	5.04 ± 0.11
E-120	0.11	6.94 ± 0.06	1.14	5.94 ± 0.06
E-121	0.09	7.05 ± 0.08	0.95	6.02 ± 0.06
E-122	0.31	6.51 ± 0.02	2.53	5.60 ± 0.05
E-123	0.11	6.96 ± 0.06	2.36	5.63 ± 0.12
E-124	1.38	5.86 ± 0.07	19.95	4.70 ± 0.00
E-125	0.13	6.87 ± 0.03	1.81	5.74 ± 0.06
E-126	0.11	6.94 ± 0.08	4.07	5.39 ± 0.33
E-127	0.36	6.45 ± 0.04	19.95	4.70 ± 0.00
E-128	0.13	6.90 ± 0.07	19.95	4.70 ± 0.00
E-129	0.42	6.37 ± 0.11	19.95	4.70 ± 0.00
E-130	0.02	7.61 ± 0.03	19.95	4.70 ± 0.00
E-131	0.03	7.52 ± 0.13	3.34	5.48 ± 0.36
E-132	1.98	5.70 ± 0.11	19.95	4.70 ± 0.00
E-133	0.14	6.85 ± 0.09	6.66	5.18 ± 0.06
E-134	0.02	7.75 ± 0.06	7.41	5.13 ± 0.15
E-135	0.01	7.96 ± 0.05	1.81	5.74 ± 0.05
E-136	0.15	6.82 ± 0.12	2.38	5.62 ± 0.05
E-137	0.17	6.77 ± 0.09	8.45	5.07 ± 0.04

E-138	0.01	8.10 ± 0.05	2.78	5.56 ± 0.12
E-139	0.02	7.74 ± 0.04	8.32	5.08 ± 0.11
E-140	0.07	7.14 ± 0.11	3.47	5.46 ± 0.23
E-141	0.07	7.13 ± 0.07	10.23	4.99 ± 0.01
E-142	0.21	6.68 ± 0.06	4.37	5.36 ± 0.04
E-143	0.01	8.03 ± 0.05	0.08	7.09 ± 0.06
E-144	1.09	5.96 ± 0.08	19.65	4.71 ± 0.00
E-145	0.20	6.70 ± 0.08	13.28	4.88 ± 0.08
E-146	0.46	6.34 ± 0.10	16.60	4.78 ± 0.06
E-147	0.31	6.50 ± 0.09	19.95	4.70 ± 0.00
E-148	0.05	7.32 ± 0.06	5.50	5.26 ± 0.14
E-149	0.07	7.18 ± 0.03	7.82	5.11 ± 0.05
E-150	0.06	7.19 ± 0.03	7.76	5.11 ± 0.10
E-151	0.10	7.01 ± 0.13	19.95	4.70 ± 0.00
E-152	0.68	6.17 ± 0.01	16.72	4.78 ± 0.04
E-153	0.04	7.42 ± 0.28	7.70	5.11 ± 0.24
E-154	1.26	5.90 ± 0.04	19.95	4.70 ± 0.00
E-155	0.02	7.71 ± 0.13	2.51	5.60 ± 0.06
E-156	0.01	7.92 ± 0.17	2.49	5.60 ± 0.02
E-157	0.05	7.34 ± 0.04	5.25	5.28 ± 0.05
E-158	0.01	8.12 ± 0.02	0.24	6.63 ± 0.14
E-159	0.08	7.10 ± 0.14	0.60	6.22 ± 0.08
E-160	0.29	6.54 ± 0.03	12.30	4.91 ± 0.08
E-161	0.04	7.45 ± 0.21	0.42	6.38 ± 0.08
E-162	0.11	6.97 ± 0.13	2.07	5.68 ± 0.04
E-163	0.04	7.41 ± 0.18	5.54	5.26 ± 0.13
E-201	0.15	6.81 ± 0.07	8.91	5.05 ± 0.14
E-202	0.01	8.16 ± 0.04	1.00	6.00 ± 0.02
E-203	0.01	8.14 ± 0.07	1.71	5.77 ± 0.11
E-204	0.01	8.04 ± 0.05	2.71	5.57 ± 0.08
E-205	0.16	6.79 ± 0.02	5.21	5.28 ± 0.08
E-206	0.84	6.08 ± 0.07	11.84	4.93 ± 0.13
E-207	0.01	7.85 ± 0.06	2.88	5.54 ± 0.25
E-208	0.22	6.66 ± 0.08	19.95	4.70 ± 0.00
E-209	3.98	5.40 ± 0.07	19.95	4.70 ± 0.00
E-210	0.07	7.16 ± 0.01	0.87	6.06 ± 0.04
E-211	0.39	6.41 ± 0.07	5.09	5.29 ± 0.06
E-212	0.63	6.20 ± 0.05	3.69	5.43 ± 0.23
E-213	0.04	7.39 ± 0.06	0.67	6.18 ± 0.05
E-214	0.30	6.53 ± 0.47	1.88	5.73 ± 0.09
E-215	0.44	6.35 ± 0.08	14.45	4.84 ± 0.04
E-216	0.16	6.79 ± 0.06	3.95	5.40 ± 0.06
E-217	0.31	6.50 ± 0.13	16.47	4.78 ± 0.05
E-218	0.62	6.21 ± 0.10	3.80	5.42 ± 0.13
E-219	0.04	7.39 ± 0.09	2.93	5.53 ± 0.10
E-220	0.08	7.07 ± 0.07	15.97	4.80 ± 0.04
E-221	0.96	6.02 ± 0.08	11.89	4.93 ± 0.05
E-222	0.03	7.55 ± 0.10	0.41	6.39 ± 0.17
E-223	0.07	7.14 ± 0.16	1.98	5.70 ± 0.12
E-224	0.01	8.05 ± 0.10	2.75	5.56 ± 0.26

E-225	0.01	7.92 ± 0.17	2.45	5.61 ± 0.37
E-226	0.01	8.13 ± 0.02	1.46	5.84 ± 0.06
E-227	0.04	7.42 ± 0.09	6.12	5.21 ± 0.06
E-228	0.09	7.06 ± 0.06	10.88	4.96 ± 0.05
E-229	0.08	7.07 ± 0.16	0.58	6.24 ± 0.04
E-230	0.05	7.28 ± 0.10	2.42	5.62 ± 0.12
E-231	0.16	6.78 ± 0.06	0.22	6.65 ± 0.23
E-232	0.01	7.92 ± 0.10	0.30	6.52 ± 0.16
E-233	0.01	8.10 ± 0.06	13.70	4.86 ± 0.09
E-234	0.07	7.17 ± 0.05	3.07	5.51 ± 0.07
E-235	0.02	7.72 ± 0.10	12.21	4.91 ± 0.02
E-236	0.02	7.73 ± 0.09	0.56	6.25 ± 0.12
E-237	0.23	6.63 ± 0.11	6.56	5.18 ± 0.13
E-238	2.07	5.68 ± 0.09	19.95	4.70 ± 0.00
E-239	0.67	6.18 ± 0.05	7.59	5.12 ± 0.09
E-240	0.08	7.08 ± 0.16	14.29	4.85 ± 0.10
E-241	0.07	7.16 ± 0.06	16.98	4.77 ± 0.04
E-242	0.01	7.84 ± 0.09	6.51	5.19 ± 0.14
E-243	0.05	7.27 ± 0.12	13.03	4.89 ± 0.13
E-244	0.05	7.27 ± 0.20	4.14	5.38 ± 0.22
E-245	0.03	7.58 ± 0.31	0.41	6.38 ± 0.13
E-246	0.19	6.71 ± 0.15	5.33	5.27 ± 0.11
E-247	0.13	6.87 ± 0.19	1.61	5.79 ± 0.23
F-101	1.49	5.83 ± 0.10	2.29	5.64 ± 0.29
F-102	0.63	6.20 ± 0.13	2.49	5.60 ± 0.23
F-103	0.18	6.74 ± 0.10	1.12	5.95 ± 0.10
F-104	1.07	5.97 ± 0.11	1.93	5.71 ± 0.09
F-105	0.54	6.27 ± 0.13	1.10	5.96 ± 0.07
F-106	1.65	5.78 ± 0.10	15.25	4.82 ± 0.03
F-107	0.78	6.11 ± 0.07	13.49	4.87 ± 0.12
F-108	1.21	5.92 ± 0.08	0.56	6.25 ± 0.07
F-109	0.24	6.62 ± 0.11	0.58	6.24 ± 0.11
F-110	0.26	6.59 ± 0.10	2.45	5.61 ± 0.11
F-111	2.03	5.69 ± 0.07	1.69	5.77 ± 0.03
F-112	0.08	7.07 ± 0.15	10.39	4.98 ± 0.09
F-113	0.40	6.40 ± 0.08	0.21	6.67 ± 0.13
F-114	7.13	5.15 ± 0.08	1.15	5.94 ± 0.16
F-115	1.01	6.00 ± 0.10	3.34	5.48 ± 0.08
F-116	8.71	5.06 ± 0.21	4.27	5.37 ± 0.08
F-117	0.31	6.51 ± 0.04	9.55	5.02 ± 0.03
F-118	0.03	7.52 ± 0.22	0.84	6.07 ± 0.05
F-119	1.69	5.77 ± 0.01	6.87	5.16 ± 0.03
F-120	8.91	5.05 ± 0.09	3.41	5.47 ± 0.09
F-121	0.93	6.03 ± 0.21	19.95	4.70 ± 0.00
F-122	11.09	4.96 ± 0.04	1.19	5.93 ± 0.02
F-123	0.95	6.02 ± 0.17	0.99	6.00 ± 0.09
F-124	11.13	4.95 ± 0.06	5.67	5.25 ± 0.08
F-125	4.43	5.35 ± 0.10	2.65	5.58 ± 0.11
F-126	0.40	6.40 ± 0.12	3.89	5.41 ± 0.12
F-127	0.17	6.77 ± 0.19	3.60	5.44 ± 0.06

F-128	2.38	5.62 ± 0.14	19.95	4.70 ± 0.00
F-129	0.22	6.67 ± 0.30	13.28	4.88 ± 0.05
F-130	0.56	6.25 ± 0.25	7.94	5.10 ± 0.01
F-131	3.26	5.49 ± 0.03	17.51	4.76 ± 0.03
F-132	5.93	5.23 ± 0.13	8.84	5.05 ± 0.14
F-133	0.60	6.22 ± 0.11	4.20	5.38 ± 0.12
F-134	0.29	6.54 ± 0.12	2.78	5.56 ± 0.25
F-135	0.23	6.64 ± 0.21	8.71	5.06 ± 0.11
F-136	0.45	6.35 ± 0.04	19.95	4.70 ± 0.00
F-137	0.10	6.98 ± 0.24	2.57	5.59 ± 0.34
F-138	0.03	7.50 ± 0.08	2.78	5.56 ± 0.25
F-139	0.08	7.08 ± 0.12	15.97	4.80 ± 0.06
F-140	0.40	6.40 ± 0.08	2.14	5.67 ± 0.10
F-141	0.06	7.25 ± 0.09	13.70	4.86 ± 0.05
F-142	0.35	6.45 ± 0.38	11.31	4.95 ± 0.14
F-143	0.22	6.66 ± 0.15	13.39	4.87 ± 0.06
F-144	2.61	5.58 ± 0.12	17.78	4.75 ± 0.03
F-145	2.42	5.62 ± 0.07	7.88	5.10 ± 0.17
F-146	0.78	6.11 ± 0.08	16.34	4.79 ± 0.05
F-147	0.32	6.49 ± 0.28	2.67	5.57 ± 0.08
F-148	0.02	7.67 ± 0.11	4.27	5.37 ± 0.13
F-149	0.07	7.13 ± 0.17	15.14	4.82 ± 0.07
F-150	0.17	6.77 ± 0.13	5.96	5.23 ± 0.15
F-151	0.24	6.62 ± 0.27	19.95	4.70 ± 0.00
F-152	1.57	5.80 ± 0.10	13.28	4.88 ± 0.06
F-153	0.33	6.48 ± 0.13	17.65	4.75 ± 0.03
F-154	0.08	7.11 ± 0.18	10.80	4.97 ± 0.15
F-155	0.17	6.76 ± 0.18	12.69	4.90 ± 0.07
F-156	0.10	7.00 ± 0.12	1.55	5.81 ± 0.23
F-157	0.10	7.01 ± 0.17	16.85	4.77 ± 0.04
F-158	0.26	6.59 ± 0.12	6.76	5.17 ± 0.27
F-159	2.12	5.67 ± 0.13	7.13	5.15 ± 0.26
F-160	2.06	5.69 ± 0.14	6.36	5.20 ± 0.21
F-161	1.50	5.83 ± 0.19	8.19	5.09 ± 0.09
F-162	4.95	5.31 ± 0.10	18.48	4.73 ± 0.02
F-163	0.14	6.85 ± 0.10	12.98	4.89 ± 0.07
F-201	1.18	5.93 ± 0.13	0.44	6.36 ± 0.40
F-202	0.18	6.75 ± 0.02	1.00	6.00 ± 0.04
F-203	0.48	6.32 ± 0.14	2.91	5.54 ± 0.05
F-204	0.21	6.68 ± 0.10	1.20	5.92 ± 0.07
F-205	0.55	6.26 ± 0.22	1.88	5.73 ± 0.13
F-206	19.95	4.70 ± 0.00	4.61	5.34 ± 0.08
F-207	0.69	6.16 ± 0.05	1.70	5.77 ± 0.30
F-208	0.46	6.33 ± 0.06	10.15	4.99 ± 0.09
F-209	0.24	6.61 ± 0.34	16.60	4.78 ± 0.06
F-211	7.64	5.12 ± 0.09	7.70	5.11 ± 0.11
F-212	0.65	6.19 ± 0.11	3.34	5.48 ± 0.07
F-213	0.83	6.08 ± 0.06	0.53	6.27 ± 0.17
F-214	4.33	5.36 ± 0.11	0.94	6.03 ± 0.09
F-215	2.42	5.62 ± 0.13	4.27	5.37 ± 0.15

F-216	2.59	5.59 ± 0.14	8.38	5.08 ± 0.09
F-217	2.69	5.57 ± 0.03	8.00	5.10 ± 0.07
F-218	2.26	5.65 ± 0.05	12.59	4.90 ± 0.06
F-219	0.23	6.64 ± 0.12	1.93	5.71 ± 0.16
F-220	0.14	6.86 ± 0.17	10.31	4.99 ± 0.17
F-221	2.49	5.60 ± 0.07	17.78	4.75 ± 0.04
F-222	0.02	7.62 ± 0.10	2.57	5.59 ± 0.11
F-223	1.37	5.86 ± 0.07	3.02	5.52 ± 0.07
F-224	0.05	7.29 ± 0.10	1.95	5.71 ± 0.29
F-225	0.03	7.46 ± 0.16	0.46	6.34 ± 0.02
F-226	0.05	7.34 ± 0.07	0.24	6.62 ± 0.09
F-227	0.03	7.48 ± 0.13	0.44	6.36 ± 0.04
F-228	0.04	7.35 ± 0.11	15.85	4.80 ± 0.06
F-229	0.29	6.54 ± 0.33	19.50	4.71 ± 0.01
F-230	2.60	5.59 ± 0.05	6.71	5.17 ± 0.16
F-231	10.00	5.00 ± 0.15	19.95	4.70 ± 0.00
F-232	1.71	5.77 ± 0.11	14.02	4.85 ± 0.09
F-233	6.61	5.18 ± 0.06	1.00	6.00 ± 0.28
F-234	2.26	5.65 ± 0.12	8.64	5.06 ± 0.21
F-235	0.08	7.07 ± 0.10	2.40	5.62 ± 0.09
F-236	0.07	7.13 ± 0.20	10.15	4.99 ± 0.12
F-237	0.15	6.82 ± 0.07	2.61	5.58 ± 0.11
F-238	0.26	6.59 ± 0.15	4.94	5.31 ± 0.14
F-239	1.38	5.86 ± 0.20	12.88	4.89 ± 0.06
F-240	0.04	7.37 ± 0.27	7.76	5.11 ± 0.18
F-241	0.25	6.60 ± 0.03	11.48	4.94 ± 0.14
F-242	0.09	7.06 ± 0.17	10.63	4.97 ± 0.06
F-243	0.11	6.98 ± 0.18	7.70	5.11 ± 0.17
F-244	9.77	5.01 ± 0.06	10.39	4.98 ± 0.13
F-245	0.48	6.32 ± 0.15	3.09	5.51 ± 0.06
F-246	6.76	5.17 ± 0.04	18.20	4.74 ± 0.02
F-247	0.68	6.17 ± 0.12	2.82	5.55 ± 0.25

Table S2. 340758+ and 2002-96+++ strain responses against the selected 5-NI compounds EC_{50} and pEC_{50} values with standard errors shown.

	T. foetus 347058+		*T. foetus* 2002-96+++	
Compounds	**EC_{50}(µM)**	**pEC_{50}**	**EC_{50}(µM)**	**pEC_{50}**
Mz	0.65	6.19 ± 0.02	0.70	6.15 ± 0.06
Rz	1.42	5.85 ± 0.10	4.02	5.40 ± 0.06
A-210	3.11	5.51 ± 0.06	2.59	5.59 ± 0.08
A-219	1.20	5.92 ± 0.12	0.70	6.15 ± 0.16
A-220	0.47	6.32 ± 0.17	1.27	5.90 ± 0.12
A-222	1.22	5.91 ± 0.06	1.50	5.82 ± 0.05
A-226	0.88	6.06 ± 0.05	1.08	5.97 ± 0.09
B-103	2.00	5.70 ± 0.11	2.11	5.68 ± 0.19
B-127	0.72	6.14 ± 0.14	0.86	6.07 ± 0.16
B-128	2.67	5.57 ± 0.10	1.93	5.71 ± 0.16
B-129	4.14	5.38 ± 0.08	3.69	5.43 ± 0.19
B-131	2.24	5.65 ± 0.07	1.79	5.75 ± 0.11
B-134	1.30	5.89 ± 0.06	1.92	5.72 ± 0.16
B-135	1.10	5.96 ± 0.07	1.25	5.90 ± 0.13
B-140	0.98	6.01 ± 0.11	0.37	6.43 ± 0.10
B-141	1.38	5.86 ± 0.14	1.19	5.92 ± 0.12
B-148	0.41	6.38 ± 0.13	1.35	5.87 ± 0.14
B-152	1.27	5.90 ± 0.19	1.09	5.96 ± 0.21
B-156	0.39	6.41 ± 0.11	0.46	6.33 ± 0.16
B-157	5.89	5.23 ± 0.12	11.48	4.94 ± 0.10
B-158	1.00	6.00 ± 0.07	1.24	5.91 ± 0.10
B-161	3.00	5.52 ± 0.10	3.74	5.43 ± 0.08
B-162	14.13	4.85 ± 0.08	19.35	4.71 ± 0.01
B-163	1.75	5.76 ± 0.13	2.53	5.60 ± 0.14
B-207	1.82	5.74 ± 0.05	1.83	5.74 ± 0.07
B-233	1.35	5.87 ± 0.03	0.96	6.02 ± 0.09

B-235	4.20	5.38 ± 0.04	3.14	5.50 ± 0.10
B-236	0.78	6.11 ± 0.08	0.99	6.01 ± 0.10
B-237	1.03	5.99 ± 0.07	1.10	5.96 ± 0.16
B-243	0.65	6.19 ± 0.14	0.88	6.05 ± 0.06
B-244	4.17	5.38 ± 0.06	5.01	5.30 ± 0.08
B-245	2.20	5.66 ± 0.10	1.75	5.76 ± 0.09
B-246	2.69	5.57 ± 0.13	5.05	5.30 ± 0.12
B-247	0.78	6.11 ± 0.12	1.41	5.85 ± 0.07
C-101	1.57	5.80 ± 0.09	0.97	6.01 ± 0.06
C-102	2.49	5.60 ± 0.10	3.02	5.52 ± 0.05
C-111	0.95	6.02 ± 0.07	1.10	5.96 ± 0.10
C-112	1.43	5.84 ± 0.12	2.69	5.57 ± 0.11
C-116	2.59	5.59 ± 0.09	2.82	5.55 ± 0.11
C-138	0.61	6.21 ± 0.08	0.47	6.32 ± 0.07
C-139	0.63	6.20 ± 0.08	0.50	6.30 ± 0.12
C-141	0.90	6.05 ± 0.05	0.72	6.14 ± 0.19
C-143	1.04	5.98 ± 0.06	0.97	6.01 ± 0.07
C-158	0.72	6.14 ± 0.06	0.48	6.32 ± 0.11
C-161	2.12	5.67 ± 0.02	1.86	5.73 ± 0.10
C-202	1.22	5.91 ± 0.08	1.00	6.00 ± 0.12
C-203	0.69	6.16 ± 0.10	0.44	6.36 ± 0.04
C-204	0.50	6.30 ± 0.10	0.32	6.50 ± 0.24
C-207	0.47	6.32 ± 0.08	0.64	6.19 ± 0.08
C-219	0.71	6.15 ± 0.12	0.62	6.21 ± 0.07
C-222	0.34	6.47 ± 0.09	0.62	6.21 ± 0.03
C-224	0.83	6.08 ± 0.12	1.45	5.84 ± 0.07
C-225	2.07	5.68 ± 0.09	2.51	5.60 ± 0.15
C-226	1.06	5.97 ± 0.08	0.85	6.07 ± 0.11
C-227	0.76	6.12 ± 0.11	1.83	5.74 ± 0.09
C-228	1.10	5.96 ± 0.13	0.81	6.09 ± 0.14

C-231	3.95	5.40 ± 0.07	4.27	5.37 ± 0.08
C-233	1.63	5.79 ± 0.12	1.12	5.95 ± 0.11
C-241	0.87	6.06 ± 0.24	1.03	5.99 ± 0.21
C-243	0.50	6.30 ± 0.10	0.40	6.39 ± 0.18
C-245	1.35	5.87 ± 0.11	1.29	5.89 ± 0.09
E-113	0.58	6.24 ± 0.07	0.55	6.26 ± 0.07
E-120	1.81	5.74 ± 0.08	1.50	5.82 ± 0.11
E-121	2.95	5.53 ± 0.05	2.51	5.60 ± 0.04
E-143	0.58	6.24 ± 0.12	0.69	6.16 ± 0.10
E-158	0.62	6.21 ± 0.12	0.67	6.17 ± 0.09
E-159	1.70	5.77 ± 0.03	1.30	5.89 ± 0.08
E-161	1.17	5.93 ± 0.10	1.22	5.91 ± 0.13
E-202	0.74	6.13 ± 0.14	0.77	6.11 ± 0.05
E-210	1.02	5.99 ± 0.12	3.02	5.52 ± 0.04
E-213	1.30	5.89 ± 0.04	1.55	5.81 ± 0.05
E-216	4.40	5.36 ± 0.05	5.21	5.28 ± 0.03
E-218	9.77	5.01 ± 0.04	11.31	4.95 ± 0.04
E-222	0.93	6.03 ± 0.08	0.74	6.13 ± 0.11
E-229	1.14	5.94 ± 0.06	1.35	5.87 ± 0.10
E-231	3.95	5.40 ± 0.06	3.29	5.48 ± 0.09
E-232	0.86	6.06 ± 0.04	0.80	6.10 ± 0.09
E-236	0.76	6.12 ± 0.13	0.75	6.12 ± 0.17
E-245	1.33	5.88 ± 0.13	1.82	5.74 ± 0.12
F-103	1.11	5.95 ± 0.08	1.25	5.90 ± 0.10
F-105	7.03	5.15 ± 0.12	5.54	5.26 ± 0.08
F-108	13.08	4.88 ± 0.09	12.12	4.92 ± 0.07
F-109	7.76	5.11 ± 0.06	7.19	5.14 ± 0.06
F-113	16.47	4.78 ± 0.05	15.73	4.80 ± 0.03
F-114	19.95	4.70 ± 0.00	19.95	4.70 ± 0.00
F-118	5.98	5.22 ± 0.06	6.76	5.17 ± 0.12

F-122	19.95	4.70 ± 0.00	19.95	4.70 ± 0.00
F-123	11.48	4.94 ± 0.14	19.95	4.70 ± 0.00
F-202	1.41	5.85 ± 0.06	2.07	5.68 ± 0.05
F-207	7.19	5.14 ± 0.08	10.31	4.99 ± 0.04
F-213	8.51	5.07 ± 0.07	9.55	5.02 ± 0.04
F-214	17.65	4.75 ± 0.03	19.95	4.70 ± 0.00
F-225	0.79	6.10 ± 0.07	0.71	6.15 ± 0.08
F-226	0.84	6.07 ± 0.14	0.62	6.21 ± 0.09
F-227	1.19	5.92 ± 0.09	1.42	5.85 ± 0.03
F-230	17.38	4.76 ± 0.03	19.95	4.70 ± 0.00