Anti-Tuberculosis Drug Discovery by Bioluminescence-Based Screening of a Protein Kinase Inhibitor Library

Honors Research Thesis

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by

Aleksandra Adamovich

The Ohio State University
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Project Advisors: Professor Larry Schlesinger, Department of Microbial Infection & Immunity and Microbiology
Professor Abul Azad, Department of Microbial Infection & Immunity
Summary with Background:

Tuberculosis (TB), an infection caused by the bacterial pathogen *Mycobacterium tuberculosis* (*M.tb*), is an ongoing global concern. Billions of people are infected with TB and millions die each year, and the mismanagement of antibiotics has led to the emergence and greater prevalence of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M.tb* in recent years. Treatment of TB is already a long, complicated process, and drug-resistant forms of the disease are even more difficult to treat. The prevalence of TB worldwide, in combination with the threat posed by drug-resistant strains, has led to a greater demand for finding new anti-TB drugs. In order to identify compounds which could serve as the basis for new anti-TB drugs, a library of putative protein kinase inhibitors was tested for inhibiting *M.tb* growth. Protein kinases in bacterial pathogens, including *M.tb*, have been shown to be promising targets for new drugs because of their essentiality for bacterial growth and pathogenesis. A library of 3000 putative kinase inhibitors from the ChemBridge Corporation was first screened by bioluminescence assay against *Mycobacterium smegmatis*, a non-virulent model organism for *M.tb*, in order to identify compounds which could successfully inhibit the growth of *M.tb*. After screening against a *M. smegmatis* reporter strain expressing luciferase, nine compounds were found which significantly inhibited its growth, as indicated by lower relative luminescence units (RLUs) over time. These nine compounds were tested in a similar fashion for their effects on *M.tb* growth in both in-vitro broth culture and inside human macrophages. From this bio-screening analysis, two compounds, 14/C5 (ChemBridge ID# 7683885) and 17/G7 (ChemBridge ID# 7691166), were identified as having the most effect on *M.tb* growth and thus considered for further development as new anti-TB drugs.
Introduction:

*Tuberculosis and its causative agent:* Tuberculosis (TB), a bacterial infection caused by *Mycobacterium tuberculosis* (*M.tb*), is one of the leading causes of death in the world due to a single infectious disease, second only to HIV/AIDS (26). About one-third of the world’s population is infected with latent TB as well (26). In 2011, 8.7 million new cases of TB and 1.4 million deaths due to TB were reported, primarily in Africa and Asia (8).

*M.tb* is an obligate, intracellular, non-motile bacillus that primarily infects humans. The bacterium is also known for its lipid-rich cell wall, which is impermeable to most dyes. *M.tb* also divides at an incredibly slow pace, taking 15 to 20 hours (14). Mycobacteria can be classified into non-pathogenic organisms, such as *Mycobacterium smegmatis*, which is fast growing and most often used as a laboratory model for *M.tb* research, and pathogenic organisms which cause diseases in humans and animals, such as *M.tb* and *M. bovis* (13). *M.tb* is generally transmitted via the inhalation of respiratory droplets, and grows best in the oxygen-rich tissues of the lung (14). Within the lung, *M.tb* is typically phagocytosed by alveolar macrophages, predominantly via the human macrophage mannose receptor in addition to complement receptors (22). Following phagocytosis *M.tb* generally remains within the phagosome, preventing its maturation and acidification in order to avoid being destroyed (21), but it has also been observed residing in the cytoplasm (18).

After macrophages are infected with *M.tb*, chemokines and other inflammatory mediators are released, attracting phagocytic cells and lymphocytes to the infected area to gather and eventually form structures called granulomas, which limit *M.tb* growth and host damage but also promote *M.tb* infection (6). *M.tb* continues to reside within the granuloma, adjusting its
metabolism in order to survive in a more nutrient depleted environment (21). Infected macrophages can also leave the granuloma and disseminate to other areas of the host to form new granulomas (19). Host cell defenses are able to destroy many of the infected macrophages and granulomas, but a few are retained, resulting in latent TB (so-called persister bacteria), which is more difficult to treat (25). However, about 5-10% of infections result in active disease due to immunosuppression caused by factors such as diabetes, stress, smoking, and HIV co-infection (17). Necrotic macrophages from within granulomas can release \textit{M. tb} into the extracellular environment, allowing for faster growth (19). Uncontrolled growth in the lungs, pneumonia being the most common form of disease, eventually results in death due to the destruction of cells involved in oxygen exchange and the obstruction of bronchiolar passages, but \textit{M. tb} can also cause disease in other parts of the body, such as tubercular meningitis within the brain (25).

\textbf{Treatment of TB:} There are currently 10 drugs on the market which have been approved by the Food and Drug Administration for treatment of TB, and the current preferred treatment regimen for standard active TB has two phases: the initial phase and the continuation phase. This consists of taking the first-line anti-TB agents isoniazid, rifampicin, ethambutol, and pyrazinamide for two months, followed by another four to seven months of isoniazid and rifampicin (27). Isoniazid acts by inhibiting the synthesis of mycolic acids, the long-chain fatty acids that compose a significant portion of the mycobacterial cell wall, ultimately resulting in cell death, while ethambutol targets the synthesis of cell wall glycolipids. Rifampicin inhibits bacterial RNA synthesis, and pyrazinamide disrupts the pH balance of mycobacteria (28).

As TB treatment is a long, involved process, there has been an increased demand for developing new drugs to make curing TB easier (9). In order to control the worldwide TB
epidemic, new TB drugs which could reduce the daily pill burden, shorten the duration of treatment, and treat MDR and XDR strains must be discovered (12). As noted earlier, \textit{M.tb} persistence in granuloma structures is an obstacle for treatment. The persistence of \textit{M.tb} in the granuloma is thought to be caused by the altered immune environment within the granuloma (24). The problem of bacterial persistence is a major reason for the requirement of extended drug treatment in order to cure TB (14). The drugs currently being used to treat \textit{M.tb} were all discovered before 1970, and the increases in \textit{M.tb} drug resistance have led to more emphasis on attempting to discover new anti-TB drugs (10).

In addition to new anti-TB drugs which are in the final stages of clinical development or are nearly approved, there are approximately 11 different compounds which are currently in different phases of clinical trials. These drugs are PA-824 (a nitroimidazole), OPC-67683 (a nitroimidazole), PNU-100480 (an oxazolidinone), AZD5847 (an oxazolidinone), SQ609 (a diamine derivative), SQ109 (a diamine derivative), DC-159a (a fluoroquinolone), TMC207 (a diarylquinoline), BTZ043 (a nitrophenyl derivative), DNB1 (a nitrophenyl derivative), and BDM31343 (an oxadiazole derivative) (28). These various drugs have numerous modes of action, such as inhibition of the cell wall, protein, or RNA syntheses (10). These new anti-TB drugs consist of molecules which have been specifically developed to treat TB, as well as existing drugs that have been redeveloped and repurposed (12, 15).

\textbf{Emergence of drug resistant strains:} Due to multiple, toxic and often inappropriately used antibiotics, numerous drug-resistant strains of \textit{M.tb} have emerged, leading to increased challenges towards the treatment of TB and the necessity to discover new drug candidates. \textit{M.tb} acquires drug resistance due to spontaneous mutation on the chromosome, and incomplete or improper treatment such as drug monotherapy can result in selection for drug resistant strains
(7). Strains of multi-drug-resistant (MDR) TB and extensively drug-resistant (XDR) TB, along with strains resistant to all anti-TB drugs, have been identified in recent decades. MDR TB is caused by strains of *M.tb* which are resistant to rifampicin and isoniazid, two of the most potent and therefore most important first-line drugs. XDR TB is characterized by *M.tb* strains resistant to rifampicin and isoniazid, any fluoroquinolone, and one of the three injectable drugs - capreomycin, kanamycin, or amikacin (7). MDR TB is currently treated with a combination of eight to ten drugs for 18 to 24 months, and mainly with drugs not developed for treating TB, which results in treatment failure 30% of the time. Treatment for XDR TB is even more limited because of its greater resistance. In addition, the drugs used to treat MDR and XDR TB often have deleterious side effects such as hepatotoxicity and nephrotoxicity (12).

60,000 MDR TB cases were reported in 2011, mainly in India, China, and Russia, but the World Health Organization (WHO) estimates this to be only 20% of the actual number of MDR TB cases developed (8). The growing spread of these drug-resistant strains of *M.tb* has even further increased the demand for more anti-TB drugs.

**Protein kinases as mycobacterial drug targets:** In recent years, there has been growing interest and effort in examining *M.tb* for novel drug targets in order to develop new antibiotics. Protein kinases, enzymes which transfer phosphate groups to other molecules, have garnered significant interest in the development of new anti-TB drugs (20, 23). Both eukaryotic and bacterial protein kinases play significant roles in cell regulation by activating or de-activating their targets, and their inhibition could be detrimental to the survival or virulence of the pathogen. Protein kinases have been found to be involved in the pathogenesis of numerous bacteria, including *Staphylococcus aureus, Pseudomonas aeruginosa,* and *M.tb* (3). Recent studies have indicated that eukaryotic-like serine/threonine protein kinases (STPKs) in *M.tb* are signal transducing
molecules essential for mycobacterial growth (2). STPKs consist of nine receptor-like molecules belonging to three different groups as based on homology – PknA/PknB/PknL, PknF/PknI/PknJ, and PknD/PknE/PknH – as well as two soluble enzymes, PknG and PknH (31).

Within mycobacteria, the STPKs are involved in processes such as cell division, metabolism, and cell wall biosynthesis. In fact, the mycobacterial kinases PknA and PknB have been identified as essential for mycobacterial cell growth processes (11). PknG has also been identified as a target for controlling mycobacterial infection, with evidence suggesting that it interferes with phagosome-lysosome interaction by phosphorylating host proteins and controls primary carbon and nitrogen metabolism within the bacterial cell (2, 13, 23). M.tb mutants without PknG have been observed as attenuated in immunocompetent mice, indicative of its necessity for virulence (13). Human kinases have also remained unaffected while PknG was inhibited using the kinase inhibitor drug AX20017, a tetrahydrobenzo[b]thiophene derivative, and the anti-cancer agent mitoxantrone has shown success in inhibiting PknB (4, 23). This indicates that M.tb protein kinases are sufficiently distinct enough from eukaryotic kinases that they could serve as potential anti-TB drug targets. In addition, the histidine kinases MtrA and SenX3 have been found to be essential for mycobacterial virulence in mice, and could serve as possible drug candidates as well (13). These facts have led to our central hypothesis that specific kinase inhibitor compounds will serve as a new class of anti-TB agents with potential efficacy for drug-resistant strains. In this project we have undertaken the screening and synthesis of numerous kinase inhibitor compounds in order to discover those which could successfully target and inhibit M.tb kinases.
Materials and Methods:

*Putative kinase inhibitor library:* The 3000 small molecule drug compounds used in this study are part of the KINASet library created by the ChemBridge Corporation (San Diego, CA). The compounds within the library were chosen based on the probability of binding to the ATP-binding region and allosteric sites of kinase targets using a 3D pharmacophore based query method. In order to find molecules which could mimic the adenosine portion of ATP and inhibit kinases, low-energy conformations of 5’-O-methyladenosine were used to generate three-point pharmacophores (30). These queries then searched through a set of drug-like molecules in the ChemBridge compound library in order to identify potential kinase inhibitors. The effectiveness of the query was validated *in silico* by searching the Chem-X database of known kinase inhibitors and identifying 85% of these compounds correctly (1). For this study, drugs were named based on the number of the 96-well plate containing the drug and the coordinate of the well, i.e. 35/B6 identifies a compound on plate 35, in well B6.

*In-vitro M. smegmatis screening:* Plasmid pMVhsp60+LuxABCDE was transformed into M. smegmatis via electroporation in order to produce a luminescent strain that was used for initial screening of the putative kinase inhibitor compounds (Figure 1).

*M. smegmatis* (pMV306+Lux) was cultured on Difco 7H11 media containing kanamycin (20 μg/ml) for 3 days at 37°C. A saturated starter culture was made by growing the strain in 7H9-OADC broth medium plus kanamycin with shaking for 2 days. This culture was diluted, 1:50 or 1:100, in 7H9-OADC plus kanamycin and grown with shaking for 16-18 hours to reach log phase (OD$_{600}$ = ~0.5). *M. smegmatis* culture was centrifuged for 5 minutes at 3000 rpm and resuspended in Tween-free 7H9 medium without kanamycin. The suspension was then diluted 1:100 in Tween-free 7H9 medium. The kinase inhibitor stocks (5 mM) were diluted to 0.5 mM
using Dulbecco’s Phosphate Buffered Saline (PBS) without calcium or magnesium for the purpose of multi-well-based screening of *M. smegmatis*.

Bacterial culture suspension (0.1 ml) and drug compound (0.5 mM) were dispensed together into the wells of 96-well plates to a final drug concentration of 20 or 10 μM. The plates containing the bacterial suspension in the presence of drug compounds were incubated at 37°C with shaking for 24 hours. Luminescence was measured on a GloMax 96 Microplate Luminometer (Promega) at 0, 4, 8 and 24 hours and recorded in relative luminescence units (RLUs). Compounds were determined as being promising based on lower RLU values, especially at 8 and 24 hour time points, compared to other compounds and no-drug control. Previous work in the lab has shown that decreased luminescence readings over time correlated to decreased bacterial growth (CFUs) on solid media. Two known anti-*M. smegmatis* drugs, doxycycline and ethambutol, were used as positive controls, while PBS was used as a no-drug vehicle control.

**In-vitro M.tb screening:** *M.tb* (pMV306hsp+Lux) clone, previously made in the lab, was cultured in 7H9-OADC-Tween plus kanamycin (20 μg/ml) with shaking for 7 days, and a portion of this culture was diluted (1:50 or 1:100) in the same medium and grown with shaking at 120 rpm for 5 days to an OD$_{600}$ of 0.4-0.5. The culture was centrifuged for 5 minutes at 3000 rpm and resuspended in equal volume of Tween-free 7H9 medium. The suspension was then diluted 100x in the same medium to achieve RLU values between 3,000 and 4,000. Potential compounds, based on *M. smegmatis* screening, were re-screened against *M.tb* using 10, 5, 2 and 1 μM concentrations. DMSO was used as a no-drug control; INH was used as positive control in some experiments. The drug compounds were added to diluted *M.tb* in culture tube and incubated at 37°C with shaking at 160 rpm for up to 72 hours. At time points of 0, 24, 48 and 72
hours, the luminescence of DMSO- or drug-treated bacterial suspension was measured using a single-tube Sirius Luminometer (Berthold). Values were read in RLUs.

**Intra-macrophage M.tb screening:** Human monocyte-derived macrophages (MDMs) were cultured for 12 days in RPMI media containing 10-20% autologous serum in 24-well tissue culture plates (2.0 x 10^5 cells/well) (22). The MDM monolayer was washed 3 times with warm RPMI, then infected with the *M.tb* (pMV306hsp+Lux) strain at an MOI of 2:1 (4 x 10^5 bacilli/well) in RPMI containing 2.5% serum and incubated for 2 hours, with initial shaking for 30 minutes, for bacterial uptake by macrophages. The infected monolayer was then washed 3 times with warm RPMI and repleted with RPMI containing 2.5% serum, along with either of the 2 most potential drugs, 14/C5 or 17/G7, at a 2.5 µM concentration. Plates were incubated for 72 hours, and infected macrophages were lysed at 0, 24, 48 and 72 hours, after which the luminescence of the intracellular bacterial suspension was measured in RLUs. Macrophage lysis was initiated with the addition of ice-cold sterile dH_2O containing 500 µg/ml of DNase to the monolayer, followed by shaking for 10 minutes. The resuspended pellet from the RPMI (in 7H9) was then added to the plates along with 0.25% SDS in PBS and shaken again for 10 minutes, followed by the addition of 0.25% BSA in PBS (16).

**Results:**

**In-vitro M. smegmatis screening:** From the initial set of 3000 putative protein kinase inhibitors, 35 compounds at 20 µM were identified as having a significant effect on *M. smegmatis* growth based on recording lower RLUs at 24 hours when compared to a no-drug control (Figure 2). Screening these 35 compounds twice at 20- and 10 µM identified 3/D6, 3/D9, 5/E10, 6/A2, 7/F3,
14/C5, 17/G7, 23/B6 and 35/G7 as having the lowest RLU readings for *M. smegmatis*, since they had the greatest effect on growth when compared to a no-drug control (Figure 3).

**In-vitro M.tb screening:** Screening of the above 9 promising compounds against *M.tb* identified 5/E10, 6/A2, 14/C5, 17/G7, and 35/G7 as having the lowest luminescence readings when incubated with bacteria at 10- and 5 µM with a no-drug control (Figure 4, 5). Incubation of *M.tb* with drug compounds at these concentrations produced significantly lower luminescence values than the negative control starting from the 0 hour time point (Figure 5). This could be an indication that luciferase light production was being inhibited by the test compounds. Incubation of these 5 compounds with *M.tb* at 2- and 1 µM concentrations indicated that the drug compounds 14/C5 and 17/G7 induced the lowest RLU readings from *M.tb*, while the positive control INH recorded lower RLU readings at later time points than any of the potential drug compounds (Figure 6). At these lower concentrations of the compounds, unlike at 10- and 5 µM, the RLU readings at 0 hour time points did not significantly deviate from that of the negative control (Figure 6).

**Effect on M.tb-infected macrophages:** Addition of 14/C5 and 17/G7 at 2.5 µM to an *M.tb*-infected macrophage monolayer showed that both compounds tested had lower luminescence readings than the no-drug control (Figure 7), indicating that these are the two most potent of the putative kinase inhibitor compounds screened so far in inhibiting *M.tb* growth. Inspection via inverted phase microscopy of macrophage monolayers incubated with these compounds showed no indication that the monolayer was destroyed (data not shown).
Discussion:

In order to find potential drug compounds for TB treatment, a small molecule library of 3000 putative kinase inhibitors from the ChemBridge Corporation, selected based on their probability of binding to kinase sites, was initially screened against the non-pathogenic mycobacterial species *M. smegmatis*, the organism commonly used in the laboratory as a fast-growing model for *M.tbc. From these 3000 compounds, an initial screen at 20 µM found 35 compounds which significantly affected the growth of a bioluminescent reporter strain of *M. smegmatis* (Figure 2). These 35 compounds were re-screened at 20- and 10 µM concentrations, resulting in the identification of nine compounds which most significantly affected the growth of *M. smegmatis* (Figure 3).

These nine compounds which had showed the most impact on *M. smegmatis* growth were subsequently tested in-vitro for their ability to inhibit the growth of *M.tbc* at 10- and 5 µM concentrations, in a similar fashion to the *M. smegmatis* screening (Figure 4). Based on the RLU values recorded from *M.tbc*, the number of potential compounds was further narrowed to five (Figure 5). These five compounds were then tested for their ability to inhibit *M.tbc* growth at 2- and 1 µM concentrations (Figure 6). The two top hit compounds, 14/C5 and 17/G7, were identified as the best at inhibiting *M.tbc* growth because they possessed the lowest RLU readings (Figure 6).

The observation of the drug compounds producing low RLU readings compared to negative control from the initial 0 hour reading at higher concentrations (10- and 5 µM) could indicate that the compounds are in some way interfering with luminescence rather than growth (Figure 5), as positive controls such as doxycycline took four hours to produce substantially lower RLU values when incubated with *M. smegmatis* (Figure 3). If these molecules affected
luminescence, determining growth inhibition of *M. smegmatis* and *M.tb* based on RLU readings could lead to an incorrect interpretation of bacterial growth. Thus, these results need to be verified by performing colony forming unit (CFU) assay in order to rule out the possibility of these test compounds interfering with the luminescence production by mycobacteria. This experiment is underway.

These two compounds were subsequently tested on *M.tb*-infected macrophages at a 2.5 µM concentration in order to determine whether these compounds could affect the growth of intracellular *M.tb*, as the bacilli typically remain located within macrophages (14). Lower RLU values were recorded from *M.tb*-infected macrophages incubated with drug compound as compared to control DMSO-treated infected macrophages, indicative of the possibility that both compounds were able to inhibit the growth of *M.tb* residing within macrophages (Figure 7). Although the macrophage toxicity assay upon exposure to the test compounds alone prior to this infection experiment was not done in this project, the infected macrophage cell monolayer was not destroyed by the presence of these compounds at 2.5 µM as indicated by inverted phase microscopy, suggesting that these compounds are not toxic to host cells at this concentration.

These results demonstrate that the putative kinase inhibitors from ChemBridge, 14/C5 (ID #7683885) and 17/G7 (#7691166) (Figure 8), appear to have the ability to significantly inhibit the growth of *M.tb* both in-vitro and within macrophages, making them potential anti-TB drug candidates. These two compounds share little structural similarity aside from the fact that they are both acetamides. In addition, no information has been found in the literature to show any similarities between either of these two structures and any drugs which are used or being developed to treat TB, indicating that these are novel structures for anti-TB drug research.
While these results are promising, further research and analysis is required into order to create the most effective drugs from these compounds. Modification of the side chains on the scaffolds could result in more efficient and potent compounds. The mode of action that these compounds take in affecting *M. tb* and *M. smegmatis* growth must also be determined – the library consists of putative protein kinases, so it must still be proven whether these compounds are protein kinases. The luminescence output from bacteria treated with 14/C5 and 17/G7 also depended on the species – while 17/G7 had been indicated as a very promising compound from the beginnings of testing against *M. smegmatis*, 14/C5 had a more significant effect on the luminescence produced by *M. tb* than on that of *M. smegmatis*. Although *M. smegmatis* is a model organism for *M. tb*, these results appear to indicate that 14/C5 is affecting a component of the organism which is more essential for *M. tb* growth than it is for that of *M. smegmatis*.

The significant effect of 14/C5 and 17/G7 on the luminescence produced by intracellular *M. tb* is another promising result. The observation that these compounds did not destroy the macrophage cell monolayer at a concentration of 2.5 μM suggests they are not cytotoxic, but a standard cytotoxicity assay (e.g., LDH assay) should be done using higher concentrations of the compound up to 20 μM. The effect of these compounds on the host must be studied more extensively. Protein kinases are found in humans as well as in bacteria, and if human protein kinases possess homologous target sites to those utilized by the compounds it could adversely affect the host. The efficacy of 14/C5 and 17/G7 in tests with a cell monolayer show these compounds currently have promise, but much more work on structural modifications, stability, the potency of these compounds and their cell biological response must be done in order to identify whether they are suitable candidates for anti-TB drugs.
Other issues, such as whether these compounds could successfully enter into the host tissue \textit{in vivo} and deliver drug compounds to the correct location, currently remain unanswered and are further off into the process of drug development than the contents of this project. The encapsulation of anti-TB drugs into liposomes has proven promising for rifampicin drug delivery into alveolar macrophages, and this method could serve as a mode of delivery for future compounds (29). The average time it takes to develop a new drug is 15 years from start to finish, and exhaustive work must be done in order to determine how individuals and drug compounds may interact (5). While this study cannot address all of these concerns, it has identified two potential compounds which could serve as anti-TB drug candidates for continuing this lengthy process and might eventually save millions of human lives from this pandemic scourge.
**Figures:**

![Figure 1](image)

**Figure 1.** Generation of a luminescent *M. smegmatis* reporter strain by introducing a luciferase-expressing plasmid. (A) A map of the plasmid. (B) Confirmation of 5 selected *M. smegmatis* transformant clones by PCR on boiled lysates, using LuxA-specific primers. M, DNA size marker; -, No DNA template; +, Plasmid DNA positive control.
Figure 2. Overview of the primary screening of the putative kinase inhibitor library against *M. smegmatis* (percent inhibition of RLUs). 3000 compounds were screened and 35 compounds were selected (shown here) for re-screening based on their abilities to maximally inhibit mycobacterial growth at 24 hour readings. Growth inhibition was determined by monitoring the luminescence in RLUs of *M. smegmatis* incubated with test drug compound and *M. smegmatis* incubated with no-drug (only PBS as vehicle) control.
**Figure 3.** Selection of 7 promising drug compounds following re-screening of the top-hit 35 compounds identified during initial screening against *M. smegmatis* (Figure 2). Graphs show the *M. smegmatis* growth inhibition profile by 7 compounds at 20- and 10 μM concentrations, respectively, during 0, 4, 8 and 24 hours of incubation. The mycobacterial growth was recorded in terms of RLU values obtained from exposure to test drug, a positive control (doxycycline), and no-drug control (only PBS).
Figure 4. In-vitro screening of 9 promising drug compounds, as identified during primary screening with *M. smegmatis* (Figure 2), at 10- and 5 μM concentrations against *M.tb* over a 72 hour period. Growth was measured in RLUs at 0, 24, 48 and 72 hour time periods and compared to a no-drug (DMSO vehicle) control.
**Figure 5.** Graphs showing the effectiveness of the 5 top-hit drug compounds (selected from screening in Figure 4) against *M. tb* growth, at 10- and 5 µM concentrations, over a 72 hour period. Growth was measured in RLUs at 0, 24, 48 and 72 hour time periods and compared to DMSO vehicle control.
**Figure 6.** Graphs showing the effectiveness of the 5 top-hit drug compounds (selected from screening in Figure 4) against *M. tb* growth, at 2- and 1 µM concentrations, over a 72 hour period. Growth was measured in RLUs at 0, 24, 48 and 72 hour time periods, and compared to isoniazid (INH) as positive control and DMSO as vehicle control.
Figure 7. Intra-macrophage growth assay of *M.tb* after exposure to the 2 best drug compounds, 14/C5 and 17/G7, at a 2.5 µM concentration over a 72 hour period. Growth was measured in RLUs at 0, 24, 48 and 72 hours and compared to a no-drug control (DMSO).
**Figure 8.** The structures of the 2 putative kinase inhibitor compounds which most effectively inhibited *M.tb* growth.

**14/C5 (# 7683885)**  
N-(3-nitrophenyl)-2-(1-oxo-2(1H)-phthalazinyl)acetamide

**17/G7 (# 7691166)**  
N-[4-(4-morpholinosulfonyl)phenyl]-2-(2-oxobenzo[cd]indol-1(2H)-yl)acetamide
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