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### The Decrease in Antibiotic Funding

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## ABSTRACT

Name: Gina Morgan

Major: Chemistry/Environmental Science

### TITLE

The Decrease in Antibiotic Funding

Advisor's Name: Kateri Ahrendt

Reader's Name: Nicholas Kallan

Tuberculosis (TB) is a bacterial disease treated with antibiotics. But because of the prevalence, nature, and lengthy treatment of the disease, TB is in need of new antibiotics. But, big pharma companies, who have historically been responsible for developing many of the world's critical antibiotic agents, have significantly decreased investment in antibiotic research and development (R&D). Many sectors are devoted to addressing this issue including non-profit, commercial, government, and academia. Specifically in academia and regards to TB, Regis University has pursued research in the identification of inhibitors of *Mycobacterium tuberculosis* class IIa fructose-1,6-bisphosphate aldolase (MtFBA), an essential enzyme in *Mycobacterium tuberculosis*. If successful, these inhibitors could serve as potential scaffolds for future therapeutic agents to combat TB. The antibiotic funding problem cannot be tackled without significant cooperation and collaboration. The issue should be addressed with increased collaboration between small biotech companies and big pharma, increased publicity for bacterial diseases, reevaluation of clinical trials, and inspiring the next generation of chemists to simply do science.

**THE DECREASE IN ANTIBIOTIC FUNDING: AN  
EXPLORATION OF TUBERCULOSIS AND THE  
PERPETUAL FIGHT AGAINST BACTERIA**

**A thesis submitted to  
Regis College  
The Honors Program  
In partial fulfillment of the requirements  
for Graduation with Honors**

**by  
Gina Morgan**

**May 2015**



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## INTRODUCTION

This thesis aims to address one of the most pressing needs in the fight against bacterial diseases: the development of novel antibiotics. Tuberculosis (TB) is one such disease in dire need of new antibiotics. Historically, big pharma companies such as Pfizer and AstraZeneca, have been responsible for the development of these greatly needed drugs. However, these companies have recently decreased funding in this area. This thesis is laid out in four chapters to illustrate the need for novel TB antibiotics, the antibiotic research and development (R&D) problem, ways in which the problem is helped, and specific research in a university setting.

Chapter 1 discusses tuberculosis (TB) on a global scale. It uses TB as an example of a bacterial infection in dire need of novel antibiotics to treat the infection. The chapter brings up some of the reasons for the need of new antibiotics for TB and bacterial infections as a whole.

Chapter 2 discusses the current state of the antibiotic pipeline and the problem with decreased investment in antibiotic research and development. It addresses some reasons why the pipeline is dry and the historical and current involvement of big pharma companies in the R&D of novel antibiotics. Specifically, it emphasizes big pharma's increased investment in lucrative pharmaceutical categories and decreased investment in antibiotics.

Chapter 3 discusses current avenues to address the antibiotic funding issue. The chapter is split up into four categories: commercial, non-profit, government, and academia. Within those sectors are many avenues that can help and are attempting to alleviate the problem.

Chapter 4 discusses a specific example of attempted antibiotic research in the university setting. Specifically, this is research I have done under the direction of Dr. Kateri Ahrendt at Regis University. The research is devoted to identifying inhibitors of *Mycobacterium tuberculosis* class IIa fructose-1,6-bisphosphate aldolase (MtFBA).

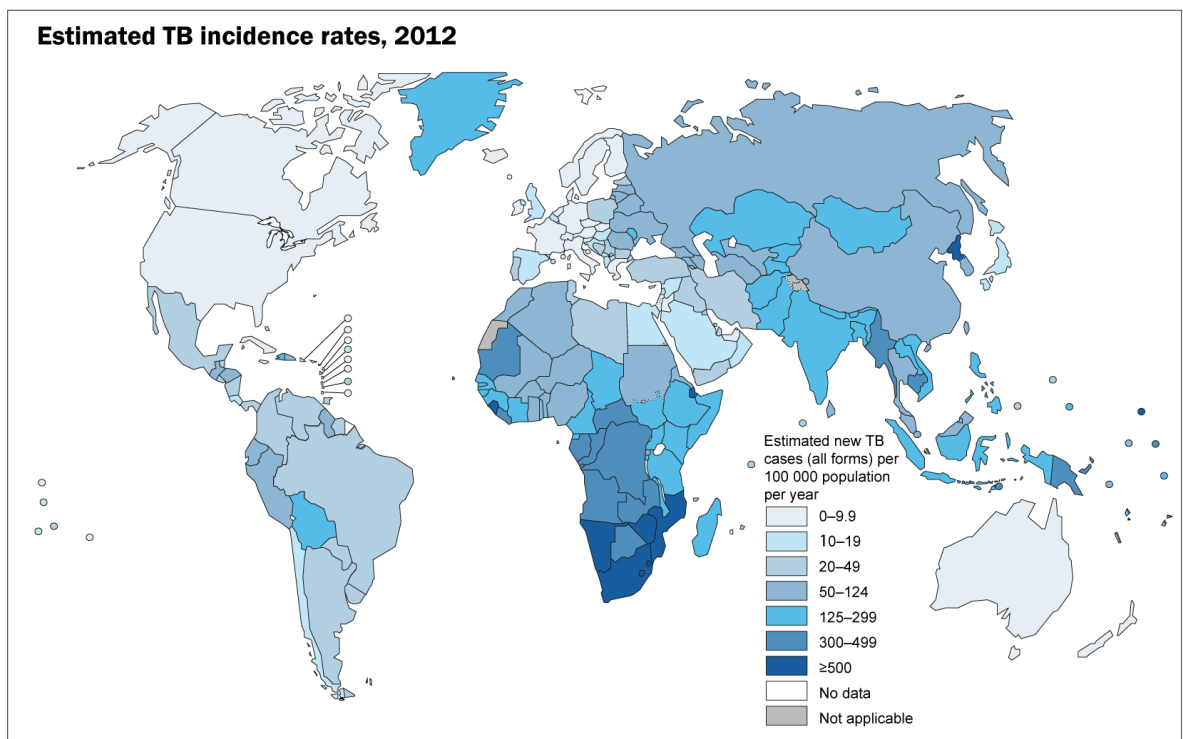
The conclusion summarizes my views on the courses of action that should be pursued most heavily in attempt to address the problem.

## **CHAPTER ONE-Tuberculosis**

Tuberculosis (TB) is a bacterial disease that affects the world on a global scale. One third of the world is infected with latent TB and the disease primarily affects developing countries, such as Africa and India. TB is one of many diseases treated with antibiotics, and if it goes untreated can kill the host. The current drug regimen recommended by the World Health Organization (WHO) involves a six month treatment with four different drugs, which if taken correctly cures the patient. However, the lengthy treatment can lead to patient compliance issues and the development of drug-resistant TB, especially in developing countries where the disease is of greater concern. New TB antibiotics are needed in order to decrease the current lengthy treatment and to better address the epidemic.

## TUBERCULOSIS ON A GLOBAL SCALE

Tuberculosis (TB) is a disease associated with poverty as over 95% of reported cases and deaths due to tuberculosis are in middle and low income countries. As illustrated in Figure 1.1, sub-Saharan Africa possesses the most new cases per population with an average of over 280 cases per 100,000 population in 2013<sup>1</sup>. The high prevalence of HIV has caused tuberculosis to rise to epidemic levels in Africa making it the home to 29% of global TB cases and 34% of TB related deaths<sup>2</sup>.



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: *Global Tuberculosis Report 2013*. WHO, 2013.



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**Figure 1.1.** World Health Organization (WHO) estimated tuberculosis incidence rates as of 2012. Adapted from: (1).

TB is second only to human immunodeficiency virus (HIV) as the greatest worldwide killer caused by a single infectious agent<sup>1</sup>. The disease accounts for about 9 million new cases and around 1.5-2 million deaths annually<sup>2</sup>. One third of the global population is infected with latent TB<sup>2</sup> and while latent TB is asymptomatic and cannot be

transmitted, an estimated 10% of these individuals will develop the active form in their lifetime<sup>1</sup>. Persons infected with HIV are 30 times more likely to develop the active disease than those without infection<sup>1</sup>. Although occurring slowly, the rate of new TB cases is decreasing each year, providing evidence that the world is making progress towards the Millennium Development Goal of halting the spread of TB by 2015<sup>1</sup>. However, TB spread was not stopped by 2015 causing the WHO to devise a plan for a post-2015 TB fight to further their original goal.

## **ANTIBIOTICS**

TB is caused from infection by the bacteria *Mycobacterium tuberculosis* (Mtb) (also known as *Tubercle bacillus*), thus rendering it one of the many diseases treated with antibiotics. Diseases treated with antibiotics pose a substantial threat of developing antibiotic resistance. Mtb is a Gram-negative bacteria<sup>A</sup> thus the bacteria pose a greater risk of developing antibiotic resistance. The Food and Agricultural Organization (FOA) defines antibiotics as “drugs of natural or synthetic origin that have the capacity to kill or to inhibit the growth of microorganisms”<sup>4</sup>. However, this definition does not capture the true aim of antibiotics. The World Health Organization (WHO) defines antibiotics as a “synonym for antibacterials used to treat bacterial infections in both people and animals”<sup>5</sup>. The FOA definition refers to antimicrobials, but the WHO acknowledges the fact that antibiotics are reserved to combat bacterial infections in human and animals, rather than combatting all diseases caused by microorganisms such as yeast, amoeba, fungi and protozoan. In the 1800’s antibiotics referred to anything that killed microorganisms, but has since been revised to incorporate the ever changing state of disease.

Although, antibiotics have successfully treated bacterial infections, bacteria can develop resistance to antibiotics, making the diseases even more difficult to control. Antibiotic resistance can occur in several ways. One way is when certain bacteria that have a natural immunity to the drug and are not killed by the first treatment with the antibiotic. The resistant bacterium grows, proliferates and creates a new drug resistant bacterial culture<sup>6</sup>. This type of resistance may be observed due to premature interruption

---

<sup>A</sup> Gram-negative bacteria have an additional outer cell membrane composed of phospholipids and lipopolysaccharides, as opposed to the single membrane of Gram-positive bacteria<sup>3</sup>. Thus, Gram-negative bacteria are harder to kill.

of the antibiotic regimen, thus ineffectively treating the disease and permitting a rise in drug-resistant bacteria<sup>1</sup>. Additionally, bacteria can gain resistance through mutations. In this case, bacteria that were once susceptible to antibiotics are no longer susceptible because their genetic code is mutated in some aspect that renders the antibiotic ineffective<sup>6</sup>.

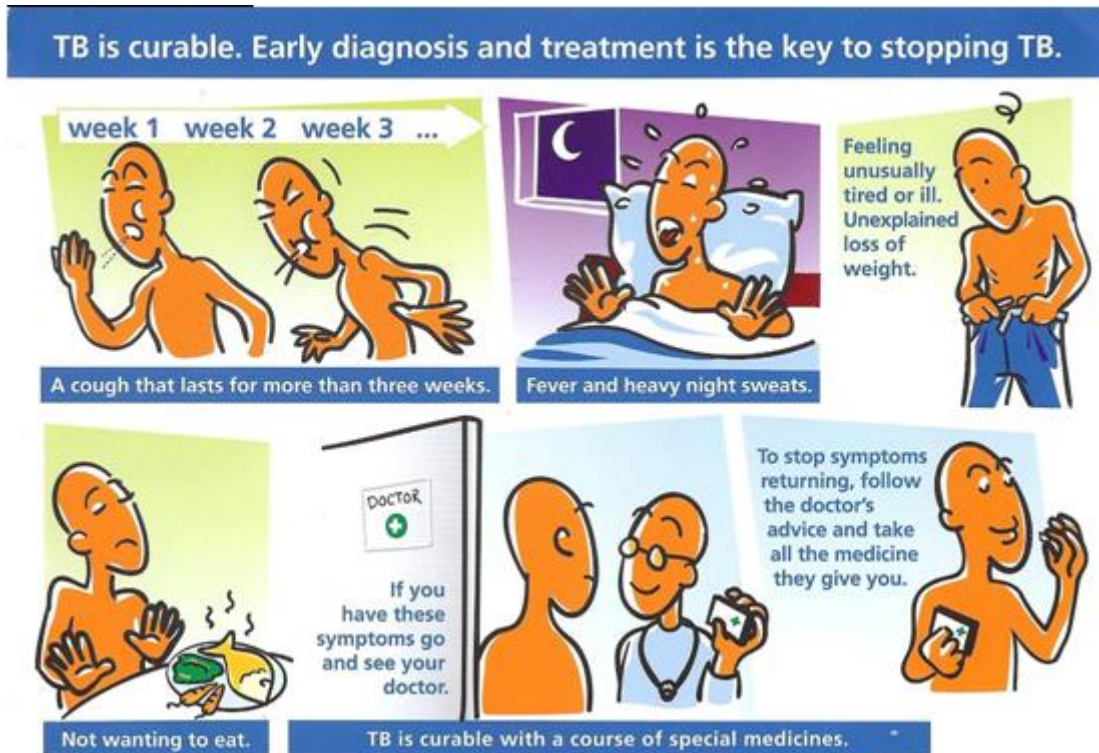
Antibiotic resistance is of great concern as diseases that were once curable by common antibiotics may no longer successfully be treated, which threatens the rise of an epidemic from a disease that was once easily controlled. In turn, tuberculosis is one such disease that has risen to epidemic levels in some areas partially due to the development of antibiotic resistance.

## **TUBERCULOSIS SPREAD AND EFFECTS**

TB is spread through the air<sup>1</sup> rather than the conventional routes of other diseases such as shaking hands, kissing, and sharing food or drink<sup>7</sup>. Once infected, the bacteria may remain dormant leading to latent TB, or spread, leading to active TB. Those with suppressed immune systems, such as HIV/AIDS patients, are at a significantly higher risk of developing the active disease<sup>1</sup>.

Active TB has several symptoms (Figure 1.2) including a bad cough lasting three weeks or longer, coughing up blood or phlegm from the lungs, chest pain, weight loss, loss of appetite, weakness, fatigue, chills, fever, and night sweats<sup>1,7</sup>. The symptoms may be mild for many months causing individuals to delay treatment potentially infecting 10-15 people within a year of infection<sup>1</sup>. If not treated, TB can be fatal. According to the World Health Organization, 2 million people die each year from TB and this number will not decrease unless TB control is strengthened<sup>1</sup>.





**Figure 1.2.** Common TB symptoms. Adapted from: Tuberculosis in India [http://microbewiki.kenyon.edu/index.php/Tuberculosis\\_in\\_India](http://microbewiki.kenyon.edu/index.php/Tuberculosis_in_India)

When infected, the bacteria most often attack the lungs but can attack any part of the body<sup>7</sup>. Pulmonary tuberculosis, affecting the lungs, is the most common form of the disease<sup>8</sup>. Pockets and cavities can form in the lungs, and the damaged areas may bleed or get infected with other bacteria and form abscesses<sup>9</sup> that lead to the formation of holes between airways in the lungs.

When a person is infected with tuberculosis, their body has a natural response to combat the disease. Activated macrophages<sup>B</sup> attempt to kill the bacteria and the body exhibits delayed-type hypersensitivity (DTH), where the body kills its own tissues so that the bacteria may be killed and prevented from replicating and harming the host<sup>10</sup>. DTH tissue damage is essential to control the infection; however, if it occurs in excess it may impair organ functions<sup>8</sup>. In a TB animal disease model, guinea pigs infected with tuberculosis died due to the DTH response, presumably because of extensive lung

<sup>B</sup> Macrophages are white blood cells that serve to fight infections in the body.

damage<sup>11</sup>. Extensive lung damage can lead to death by suffocation due to insufficient oxygen to the host<sup>8</sup>. Additionally, a rare cause of death in TB infections is a Rasmussen's aneurysm. This is the result of damage to the pulmonary artery by cavitory lesions, causing massive bleeding and ultimately death<sup>12</sup>.

Additionally, TB can affect other areas of the body including the brain and bones. For example, tubercular meningitis, occurring in the meningeal membranes of the brain<sup>C</sup>, causes death due to inflammation of the brain leading to fatal seizures and hydrocephalus<sup>8,D</sup>. Extrapulmonary<sup>E</sup> forms of tuberculosis may affect the bone and are associated with significant bone deformations and defects<sup>8</sup>. Fortunately, tuberculosis is a treatable disease and death is avoidable upon proper diagnosis and treatment.

## DIAGNOSTICS



**Figure 1.3.** Tuberculin skin test injection site and measurement. Adapted from: (9)

There are several methods used to determine whether or not an individual is infected with TB so that they may be treated before a fatality occurs. The tuberculin skin test (also known as the Mantoux test) requires a doctor to inject a small amount of tuberculin (a protein extracted from *M. tuberculosis*) into the skin. Two to three days later the doctor looks at the injection site

which, if infected, may be a raised hard or swollen area with an appearance similar to a positive allergy test. The results of the test are determined based on the size of the bump (Figure 1.3). A positive test indicates that the patient has been infected with TB; however,

---

<sup>C</sup> It is hypothesized that *M. tuberculosis* has the ability to breach the blood brain barrier by the specialized endothelial cells lining the brain microvasculature, human brain microvascular endothelial cells (HBMECs). *M. tuberculosis* triggers its own uptake into the cells indicating that the required conditions may be specific to a particular virulence<sup>13</sup>.

<sup>D</sup> Hydrocephalus is excessive fluid build-up in the brain.

<sup>E</sup> Extrapulmonary means outside of lungs.

this result does not provide information whether or not the disease is in its active or latent form<sup>7</sup>.

False-positives can occur in the Mantoux test if the patient has received the bacilli Calmette-Guerin (BCG) vaccine. The BCG vaccination is most often given to people inhabiting countries where TB is a higher threat than in certain developed countries (such as the United States). It is reserved for people with very specific requirements including children that have previously been ineffectively treated for TB and health care workers that take care of a high amount of patients infected with drug-resistant TB<sup>14</sup>.

Additionally, TB blood tests are used to determine if a patient has been infected. The test, known as the Interferon Gamma Release Assay (IGRA), measures the patient's immune response to Mtb<sup>7</sup>. A positive result indicates that the person has been infected with TB, but like the Mantoux test, it does not provide information concerning the activity of the disease.

Once TB infection has been identified in a patient, further tests are needed to determine whether or not the patient has the active form of the disease. The most common method is smear microscopy where clinical material is smeared on a glass slide and a trained professional identifies Mtb in the material. This method easily identifies highly infectious TB cases, is relatively inexpensive, and only takes a few hours to complete. However, it has a low case detection at 20-30%, thus requiring multiple repetitions and repeat visits by the patient<sup>15</sup>. Additionally, smear microscopy is unable to distinguish between drug-resistant and drug-sensitive TB.

Bacterial cultures are more sensitive than smear microscopy and successfully identify Mtb in over 80% of TB cases<sup>15</sup>, which provides a more definitive diagnosis<sup>16</sup>. However, this method still has its drawbacks. Visual detection of bacterial colonies is slow at 2-6 weeks compared to only a few hours in the smear microscopy method. Additionally, the test is expensive and is limited in developing countries that have inadequate access to resources<sup>15</sup>.

The above methods determine if a patient has active TB; however, none of the tests have the ability to identify if the disease exhibits any drug-resistant properties. Several tests have been developed that have the potential to determine this, but the tests

are still in the beginning stages, thus the timing of the tests and most cost-effective diagnostic tools are still unknown<sup>15</sup>. Nevertheless, a new two-hour test (Xpert test) that has been effective in identifying drug-resistance has been introduced across several countries<sup>17</sup>. The test analyzes TB deoxyribonucleic acid (DNA) for genetic mutations that make the bacteria resistance to certain antibiotics<sup>16</sup>.

## TB CHEMOTHERAPY

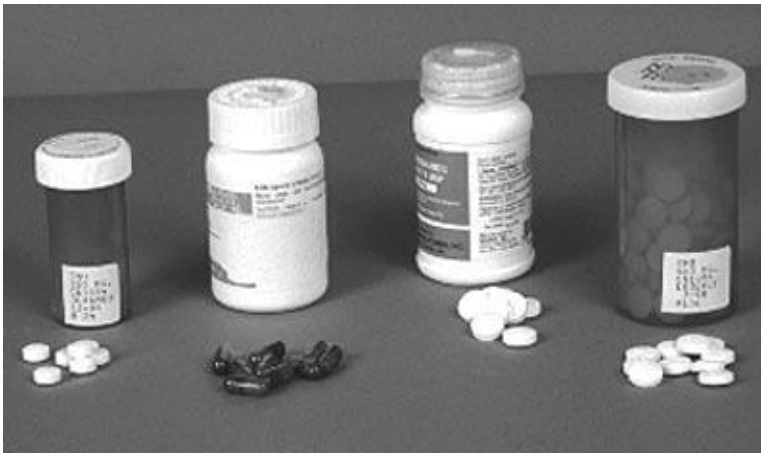
Once a positive test is confirmed, the patient begins treatment. TB chemotherapy began with the discovery of streptomycin (SM) in 1944<sup>2,18</sup> followed by the discovery of para-aminosalicylic acid (PAS) in 1946<sup>19</sup>. In 1946 the British Research Medical Council performed a randomized clinical trial of SM, proving its efficacy; however, in 1948 (a short two years later), SM resistance in pulmonary tuberculosis was observed<sup>18,20</sup>. Isoniazid (INH) was discovered in 1952, due to a 1945 discovery of nicotinamide's anti-tuberculosis activity<sup>21</sup>. The use of prothionamide (PTH), discovered in 1956, in combination with SM was observed to prevent bacterial resistance observed with treatment using only SM, giving rise to the important principle of drug combination in TB treatment<sup>2</sup>. However due to issues such as resistance, most of the mentioned drugs are not in the current treatment recommendations.

**Table 1.1.** Most common drugs used to treat TB. First-line drugs are used to treat drug-susceptible TB, the injectable second-line drugs are used to treat drug-resistant TB.

<b>Common TB drugs used</b>	
<b>First-line (used to treat drug-susceptible TB)</b>	<b>Second-line (used in addition to first-line to treat drug-resistant TB)</b>
isoniazid, rifampin, pyrazinamide, ethambutol, streptomycin, thioacetazone <sup>F</sup>	amikacin, capreomycin or kanamycin

<sup>F</sup> thioacetazone is not commonly used but can replace rifampin when there is a cost issue.

Current TB chemotherapy is intensive, requiring six months of treatment with four different antibiotics. For drug sensitive TB, the World Health Organization (WHO) recommends two months of therapy with isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) (Table 1.1, Figure 1.4), followed by an additional four months of INH and RIF treatment<sup>2</sup>. In the United States this treatment costs about \$2,000 per patient, and this regimen has been successful with a 78-95% cure rate<sup>1</sup>. In low income countries where cost is an issue, RIF can be replaced with thioacetazone or EMB, lengthening the treatment by two months but providing comparable results<sup>2</sup>.



**Figure 1.4.** Drugs used to treat drug-sensitive TB disease. From left to right isoniazid, rifampin, pyrazinamide, and ethambutol. Streptomycin (not shown) is given by injection. Adapted from: [http://textbookofbacteriology.net/tuberculosis\\_3.html](http://textbookofbacteriology.net/tuberculosis_3.html)

The four drug regimen is used in order to prevent drug resistance as each drug is designed to act on specific bacterial populations with different metabolic states. This idea serves to prevent drug resistance and improve therapy efficacy<sup>2</sup>. In most bacterial infections a small amount of bacteria remain after proper treatment and the body's immune system

is capable of killing the remaining cells. However, the human body often cannot fully eradicate the remaining *Mtb*<sup>22</sup> leaving a troublesome persister<sup>G</sup> population (dormant bacteria that are not initially eradicated with drugs). *Mtb* resides in a multitude of different environments including a highly oxygenated state in the lungs of those infected, a low oxygen state in its host (macrophage phagolysosome), a virtually anaerobic state in closed

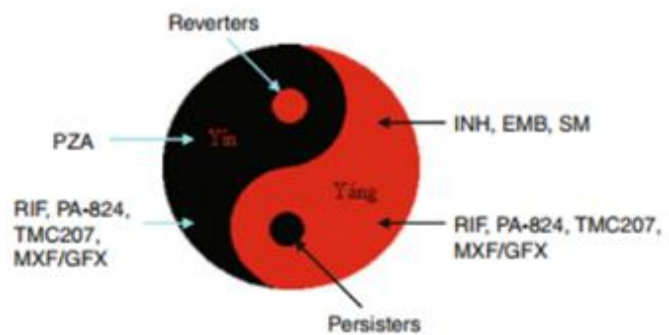
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<sup>G</sup> It is speculated that *Mtb* utilizes ManLAM (a TB virulence factor) to activate an anti-inflammatory immunosuppressive program by antigen-presenting cells that can modulate T cell responses to maintain persistence in the host<sup>22</sup>.

lesions, and an acidic state during inflammation leading to the production of bacteria with different metabolic states<sup>2</sup>.

Mtb exhibits four distinct metabolic states, all of which are affected by the different TB drugs. explained by one treatment model<sup>23</sup>. In the model, actively growing cells are mainly killed by INH, semi-dormant bacteria in an acidic environment are mainly killed by PZA, semi-dormant bacteria demonstrating spurts of active metabolism are preferentially killed by RIF, and completely dormant organisms are not killed by drugs, and thus form the persister population.

The presence of dynamic bacterial subpopulations, illustrated in Figure 1.5<sup>2</sup>, explains the need for a two phase chemotherapy. As mentioned, the first block of treatment involves INH, RIF, PZA, and EMB for the first two months and a continuation of INH and RIF for the following four months. After the first two months of treatment with all four first-line drugs, actively



**Figure 1.5.** Yin-Yang model of TB life cycle as proposed by Zhang. Demonstrates the effect of different TB drugs on TB bacterial populations. Adapted from: (2)

growing bacteria have been killed and now compose the minority of the bacterial population<sup>2</sup>, thus the majority remaining is dormant persister bacteria. Some of the dormant persisting bacteria can revert to actively growing bacteria that can be killed by INH and RIF, thus providing a reason for continuing the use of INH and RIF for an additional four months<sup>2</sup>. Similarly, this is the reason that INH is used to treat the asymptomatic latent TB. In latent TB some dormant bacteria may revert and become active, but INH kills them before they grow and proliferate and cause an active disease state. While this therapy provides a clinical cure for the disease some bacteria can remain in lesions as persisters, thus leading to a possible relapse<sup>2</sup>.

This two phase chemotherapy is designed to decrease the incidence of antibiotic resistance; however, issues such as patient compliance can lead to the development of resistance.

## **DIRECT OBSERVED TREATMENT SHORT-COURSE (DOTS)**

Because treatment is lengthy, patient compliance issues can arise, rendering further treatment ineffective due to the development of antibiotic resistance. One of the main causes of antibiotic resistance in TB is premature interruption of treatment<sup>1</sup>. As such, the WHO has developed the direct observed treatment short-course (DOTS) which aims to address the patient compliance issue and other issues at the heart of the WHO's stop TB strategy. The strategy aims to dramatically reduce the global TB burden by 2015<sup>H</sup> and support the development of new tools to prevent, detect, and treat TB<sup>1</sup>. The strategy incorporates six components, with the five main components of DOTS including:

1. Political commitment with increased and sustained financing
2. Case detection through quality assured bacteriology
3. Standardized treatment with supervision and patient support
4. Effective drug supply and management system
5. A monitoring and evaluation system and impact measurement<sup>1</sup>.

The third category on the above list incorporates ideas that aim to help control the development of future TB antibiotic resistance by addressing the causes of premature treatment interruption. With supervised treatment, patients are more likely to complete the full treatment and be cured, leading to a decrease in antibiotic resistance. In some affected persons, such as prisoners, drug users, and the mentally ill, the supervision may include direct observation of therapy (DOT) where a trained professional administers the drugs and watches the patient swallow them<sup>24</sup>. If supervision is necessary, it may be carried out in a local health-facility, the workplace, the community, or at home<sup>1</sup>. When DOT is unnecessary, supervision is context-specific and given in a patient-sensitive manner that aims to ensure cooperation on the part of the providers of medication, support, and the patient receiving it<sup>1</sup>. Support groups can also help with patient adherence, leading to a complete treatment regimen.

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<sup>H</sup>A post-2015 action plan has been created as the TB burden was not reduced to the intended extent by 2015.

As expressed earlier, TB is associated with poverty, propelling the need for proper treatment in these areas. DOTS aims to improve access to treatment so that poverty-stricken rural areas have greater access to treatment and have the ability to complete the full TB treatment regimen. DOTS intends to address the physical, financial, social, and cultural barriers that impede adequate TB treatment<sup>1</sup>. The main barrier to this treatment is the prevalence of drug-resistant TB<sup>18</sup>. Additional barriers include a governmental lack of resources, unwillingness to invest in health programs<sup>25</sup>, and a patient's lack of monetary means to pay for necessary TB treatment. In African regions especially, HIV co-infection serves as another barrier compromising the person's immune system and ability to eradicate the disease even in the presence of proper treatment<sup>18</sup>. Further roadblocks affecting African countries include declining socioeconomic conditions, populations that are at heightened vulnerability, and restraints on human resources affecting the health service sector<sup>18</sup>. In order to combat these barriers the WHO advises actions that include expanding treatment outlets in the poorest settings, providing care in closer proximity to patients, and offering heavily subsidized or free services<sup>1</sup>. Unfortunately, some developing countries have refrained from employing the advised DOTS course of treatment<sup>18</sup>.

### **DRUG-RESISTANT TB CHEMOTHERAPY**

While chemotherapy using INH (1952), RIF (1957), PZA (1952), and EMB (1962)<sup>26</sup> led to a decline in TB prevalence, strains of TB resistant to these drugs began to emerge in the in the 1980s<sup>27</sup>. There are currently three types of drug-resistant TB strains: multi-drug resistant TB (MDR-TB, resistant to at least INH and RIF), extensively drug-resistant TB (XDR-TB, resistant to INH, RIF, and any one of the fluoroquinolones or second-line injectables) (Table 1.1), and the less frequent rifampin-resistant TB (resistant to RIF; MDR-TB and XDR-TB fall into this category)<sup>1</sup>. The incidence of TB and MDR-TB decreased from 1993 to 1999, but shortly thereafter the WHO was forced to expand its definition of MDR-TB to include XDR-TB<sup>27</sup>.

Although the current TB regimen has shown success, Mtb resistance has complicated the already lengthy chemotherapy. There are two ways a person can contract MDR-TB. The first way is the development of drug-resistance due to previous inadequate treatment of drug-susceptible TB. The second way is to contract it from another person



infected with the drug-resistant TB<sup>1</sup>. It is believed that direct transmission is the most common way MDR-TB is contracted<sup>28</sup>.

The drug regimen for MDR-TB is complex and based on the patient's individual drug resistance profile. The patient undergoes drug susceptibility testing to assess which drugs their strain is resistant to. The patient is treated with reserve drugs, including the fluoroquinolones (discovered in the 1980s), and any remaining first line drugs that their particular strain is not resistant to. Therapy for MDR-TB uses five antibiotics over an 18-24 month period<sup>26</sup>, including one injectable, until bacterial cultures test negative<sup>29</sup>. Following the negative result, treatment with a minimum of three drugs is continued for an additional 9 months<sup>29</sup>. The cost of this treatment is very high, ranging from \$85,000-\$120,000 (as compared to \$2,000 for drug-susceptible TB), rendering it less accessible in developing countries. To address these high costs the WHO has proposed the DOTS-plus program for observation of MDR-TB treatment<sup>29</sup>. The program requires drug availability at a reasonable cost, a good TB program, and support for drug-resistance monitoring then the treatment may be carried out with reasonable cure rates<sup>29</sup>.

MDR-TB requires excessive treatment, often lasting for multiple years, and brings very serious side effects. Because the current treatment cannot eradicate the persisting population of bacteria, TB patients are at a higher risk of disease relapse. The main side effects include:

1. Ototoxicity (hearing loss)
2. Psychiatric disorders
3. Gastrointestinal effects
4. Arthralgia (joint pain) and arthritis
5. Seizure activity
6. Hepatitis
7. Rashes
8. Low white blood cell count
9. Peripheral neuropathy (numbness, tingling of limbs)
10. Nephrotoxicity (creatinine serum rise)
11. Hypothyroidism<sup>30</sup>.

Additionally, patients with XDR-TB are at an even higher risk. Their particular strain is resistant to the most potent first and second-line drugs. Like patients with MDR-TB, the

XDR-TB patient undergoes drug susceptibility testing and is consequently treated with additional drugs. These can include drugs not previously used in the area of treatment<sup>31</sup>, and additional Oral Bacteriostatic Second Line Agents (para-aminosalicylic acid, cycloserine, terizidone, thionamide, prothionamide)<sup>16</sup>. However, successful treatment lasts for more than 24 months and necessitates aggressive regimens with the highest tolerated doses<sup>31</sup>. The XDR-TB treatment regimen also provides similar side effects as MDR-TB treatment.

Treatment for drug-resistant TB is extremely lengthy and has detrimental side effects; however, most individuals with drug susceptible TB are cured with the six month regimen, with an estimated 22 million lives saved by the therapy. Even though the treatment has shown success, it is lengthy and uses multiple drugs which in conjunction with incorrect use of antibiotics, ineffective formulation of drugs, and premature interruption of treatment, presents a greater chance of the development of antibiotic resistance<sup>1</sup>. These, along with other reasons, propel the need for novel TB antibiotics. Unfortunately, the main discovery engines for these needed drugs are not currently keeping up with the present need for antibiotics.

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## **CHAPTER 2-Antibiotic Funding**

The current pipeline for novel antibiotics is very limited. There are few new chemical entities entering or many novel antibiotics leaving the pipeline. This can partially be attributed to big pharma's decreased investment in antibiotic research and development (R&D) in lieu of more lucrative pharmaceutical areas. In the past, big pharma has been responsible for developing some of the most successful antibiotics including the most profitable Zithromax®. However, these companies are reducing their antibiotic R&D efforts. Two of the few remaining big pharma companies with active antibiotic R&D programs, Pfizer and AstraZeneca, have recently significantly reduced or abandoned their funding for antibiotic R&D. Part of the problem can be attributed to the presence of patents. Patents are designed to promote innovation as they grant protection for an invention with a promise of return for the investor. But, with competition from generics pharmaceutical companies are further pressured into devoting funding into more profitable therapy areas.

## THE CURRENT STATE OF THE ANTIBIOTIC PIPELINE

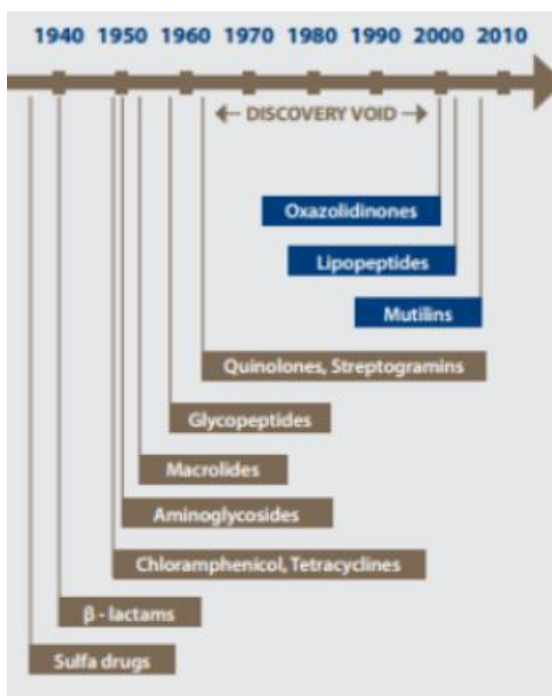
Although, there is a desperate need for novel antibiotics to treat tuberculosis and many other infectious diseases, a significant lack of development of new antibiotics is leaving the antibiotic pipeline dry<sup>1</sup>. The antibiotic pipeline includes newly synthesized compounds that are still in pre-clinical testing and clinical development<sup>2</sup>. Generally, pre-clinical drug testing uses animal models to assess potential harmful effects of the new drug. If the animal model does not show any adverse effects, then it may be assumed that the drug might be safe for human use. The drug then undergoes phase I clinical trials in healthy human volunteers. The volunteers are given the drug in a specified dose for a specified amount of time. If they do not exhibit adverse effects then the drug will be moved on to phase II and phase III trials. There is a small line between phase II and III trials, but they are both reserved for testing on humans afflicted with the disease<sup>3</sup> (Figure 2.1). These late stage trials are used to ensure drug efficacy. All potential drugs, whether they are simple anti-inflammatories or novel antibiotics, are required to enter clinical trials. After these trials, the drug may finally enter the market with the prospect of returning revenue for the discovering company.

The discovery of the sulfonamides (ex. pediazole), and  $\beta$ -lactams, (ex. penicillin), in the 1930s<sup>4</sup> initiated the “golden era” of antibiotics that lasted until the 1970s<sup>5</sup>. This age of discovery brought about seven new major classes of



**Figure 2.1.** A general outline of clinical trial phases. Adapted from: <http://scienceblog.cancerresearchuk.org/2012/05/21/international-clinical-trials-day-the-wales-cancer-trials-unit-goes-global/>

antibiotics<sup>1</sup>, as illustrated in Figure 2.2. These discoveries helped make the rampant diseases of the eighteenth and nineteenth centuries appear like a thing of the past<sup>7</sup>. In 1967 the Surgeon General stated that “it was time to close the book on infectious diseases”<sup>8</sup>. However, soon after, bacteria started to gain the upper hand. Between the 1970s and 1999, no new classes of antibiotics were discovered<sup>4,5</sup> leading to a discovery void known as the “innovative gap”<sup>9</sup> (Figure 2.2).



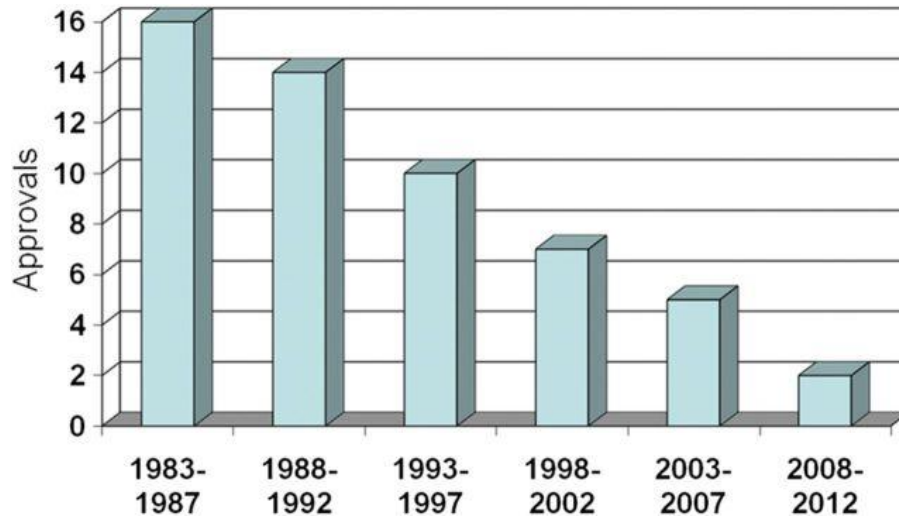
**Figure 2.2.** Between 1962 and 2000 no new major classes of antibiotics were discovered. This lack of development of new antibiotics is known as the “innovative gap”. Adapted from: (4)

Between 2000 and 2013, only 22 antibiotics were approved by the Food and Drug Administration (FDA) for market, averaging less than two new per year<sup>5</sup>, as illustrated in Figure 2.3. Within those include five new classes, three of which (oxazolidinones, lipopeptides, and pleuromutilins,) were previously reported or patented before 2000, but were marketed after the turn of the century<sup>9</sup>. Even with the very limited success in recent antibiotic R&D, the new classes of antibiotics have significant limitations in their ability to only treat Gram-positive bacteria<sup>5</sup>. Gram-negative antibiotic R&D is of great concern due

<sup>1</sup> An antibiotic class has a distinct chemical structure<sup>6</sup>.



to increased multi-drug resistance and the leaner antibiotic pipeline for these bacteria versus Gram-positive<sup>10</sup>.



**Figure 2.3.** New antibacterial agents approved by the United States Food and Drug Administration, 1983-2012, in 5 year periods. Adapted from: (21)

Scientists argue that current antibiotic drug development will not be able to keep up with the increasing antibiotic resistance among pathogens<sup>11</sup>, and according to reports by the Infectious Diseases Society of America (IDSA) and the European Centre for Disease Control the antibiotic pipeline lacks drugs with greater efficacy over other known antibiotics<sup>12</sup>. The World Health Organization has claimed that the rise in antibiotic resistance is one of the greatest threats to humankind<sup>4</sup>. Since the beginning of antibiotic use in the 1940s and 1950s, drug resistance has been present and now affects all major classes of antibiotics<sup>4</sup>. The increasing rise of antibiotic resistance and lack of antibiotics in the pipeline is a global health concern. But, antibiotic development has been slow lately for a multitude of reasons.

## THE ISSUE OF ANTIBIOTIC FUNDING

The shrunken antibiotic pipeline and decreased research and development (R&D) towards new antibiotics can largely be attributed to a lack of funding for antibiotic R&D<sup>13</sup>. Big pharma companies have been and are continuously halting funding for research of antibiotics in lieu of more lucrative pharmaceuticals, such as antidepressants, cholesterol-lowering drugs, anti-hypertensive agents, and type 2 diabetes treatments. These drugs all require chronic administration<sup>5</sup> as opposed to antibiotics that are taken for several days or weeks at maximum. This limits the market return of antibiotics as they do not need to be as much and are not purchased as frequently as other drugs. Drugs that require chronic administration have the potential to be blockbuster drugs, which have peak annual single-product sales of at least \$1 billion<sup>10</sup>. For example, even the world's most lucrative antibiotic, Pfizer's Zithromax®<sup>J</sup>, at one point profited \$2 billion annually, which is significantly lower than the biggest selling drug of all time, Pfizer's Lipitor®, which is a cholesterol lowering drug. Before its U. S. patent protection expired, Lipitor® brought in \$9 billion annually<sup>14,15</sup>. Antibiotics do not create as much revenue for developing companies for many reasons.

Market incentives have always been seen to be a driving factor for antibiotic research<sup>8</sup>. Several factors contribute to the antibiotic market that has little to return to the investor. First, like all drugs, after patent expiration generics enter the market and are able to take profit from the original developing company. Notably, the 20 year patent life causes more generics to be prescribed versus patented drugs which tells industry that even if they develop an effective antibiotic it may not be able to make a significant impact in the market. Second, in Europe specifically, public health pushes to reserve newer drugs for serious cases and treat common infections with old antibiotics. Third, the limited duration of antibiotic treatment and the curative nature of the drug (in that it is designed to cure a disease rather than alleviate symptoms) increases marketing costs for the antibiotic. Fourth, as bacteria develop resistance to the created antibiotic, the antibiotic has a shorter lifespan in the market, causing companies that invest billions of dollars into creating the

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<sup>J</sup> Zithromax® is a popular antibiotic because of its desirable dosing. The patient only has to take one pill for five-days, as compared to other antibiotics that require 2-4 pills for 10 days. Patients are more likely the regimen. In addition, it is used to treat several infections, making it appealing to prescribe for doctors.

drug to not receive full monetary benefits of their investment. Fifth, correlation between the value of the effect of antibiotics does not agree with how much the purchaser pays<sup>14</sup>. For example, ceftaroline fosamil, an antibiotic to treat community-acquired bacterial pneumonia<sup>16</sup> costs \$609 for a one-week course whereas Yervoy®, a melanoma treatment costs \$120,000 for a 12-week course (\$10,000 per week)<sup>17</sup>. It should be noted that if effective, antibiotics serve to continue a patient's life for many years to come while anticancer drugs may only prolong the patient's life for several months or years<sup>14</sup>. However, the cost of antibiotics is significantly cheaper than anticancer drugs, downplaying the effect of the antibiotic and reducing its market value. These factors, along with many others, cause antibiotics to have a limited investment return, understandably dissuading companies from devoting money into developing antibiotics<sup>14</sup>. This leads to an economic driver that is at odds with the medical and social goals of antibiotics, leading to industry abandoning antibiotic R&D<sup>10</sup>.

## **EROOM'S LAW**

The idea of Eroom's law contributes to a stagnant antibiotic pipeline even further than the market and investment perspective. Eroom's law arises from Moore's law, in fact it is Moore spelled backwards. Moore's law is an economic idea, based on the observation that the number of transistors for computers doubled every two years from 1970 to 2012<sup>18</sup> while the costs of production stayed the same or decreased. This brings greater functionality for the same cost<sup>19</sup>. The law presents itself as a forecast for societal economic growth and possible improvement of mankind<sup>19</sup>. However, pharmaceutical R&D has coined Eroom's law as an explanation for the lack of development in drug research. Contrary to a doubling of transistors every two years as observed by Moore's law, Eroom's law has observed a decrease in halves of new drugs in the market every 9 years since 1950<sup>18</sup>. The law presents the idea that powerful forces<sup>K</sup> have been able to outweigh the

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<sup>K</sup> Some of these powerful forces include the better than the Beatles problem, cautious-regulator problem, and throw money at it tendency. The better than the Beatles problem says that if people still like the Beatle's music and can download it for free, it will be very difficult to have commercial success in the music industry. The producer has to make music that is 'better than the Beatles'. This is similar for pharmaceuticals as previous blockbuster drugs are today's generics<sup>18</sup>. The back-catalogue of pharmaceuticals is increasing, thus presenting a far more complex pharmaceutical research and development process, deterring R&D in some areas, and further decreasing the economic value of drugs to be discovered<sup>18</sup>. The cautious-regulator problem says the increasing regulations, that are sometimes unnecessary significantly reduce the number of prospective drug

scientific, technological, and managerial improvements that have occurred in the past 60 years<sup>18</sup>.

## **BIG PHARMA DECREASED INVESTMENT**

Historically, large pharmaceutical companies have been responsible for developing many of the critical antibiotic agents<sup>8</sup>. But these companies have been and are continuously decreasing investment due to many of the reasons stated above. The 1960s were a very prolific time for the development of antibiotics, with the pharmaceutical industry developing the sulfonamides, penicillin, streptomycin, tetracyclines, isoniazid, macrolides, glycopeptides, cephalosprins, nalidixic acid and other classes leading up to the discovery of rifampin<sup>8</sup>. As mentioned, the innovative gap followed the 1960s. However, the 1990s saw a small resurgence from the pharmaceutical industry with the development of Pfizer's Zithromax®, Aventis's Synercid®, and Pharmacia's Zyvox®<sup>8</sup> (Table 2.1). The introduction of Zyvox® in 2001 was the first new class of antibiotics (oxazolidinones) to enter the market since rifampin in the 1960s<sup>8</sup>.

**Table 2.1.** Historical big pharma involvement in antibiotic R&D, after the innovative gap until now. Adapted from: (8)

<b>1990</b> – 50% of US and Japanese large Pharma report that they have halted or significantly decreased antibiotic discovery efforts
<b>1990</b> – Outbreak of vancomycin-resistant enterococcal infections plus increasing methicillin-resistant <i>Staphylococcus aureus</i> — some companies return
<b>1999</b> – Synercid licensed
<b>2000</b> – Roche spins off anti-infective discovery
<b>2000</b> – Zyvox, a member of the first new antibiotic class in 35 years, is licensed
<b>2001/2</b> – BMS, Lilly and Wyeth halt anti-infective discovery; Glaxo SmithKline downsizes anti-infective effort. Aventis announces intention to spin-off their anti-infective group
<b>2011</b> – Pfizer reduces, then abandons antibiotic R&D
<b>2013</b> – Astrazeneca reduces antibiotic R&D

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candidates. The throw money at it tendency is the tendency to add human resources and other resources to R&D, leading to a rise in R&D spending. People then try to reduce the costs of R&D which may limit productivity<sup>18</sup>.

**Table 2.2.** Big pharma antibacterial pipeline (anti Gram-negative, anti Gram-positive). Adapted from: (21)

Company	Since 1998	Phase 2/3
Abbott Laboratories	0	0
AstraZeneca	0	2
Bayer	0	0
GlaxoSmithKline	0	1
Lilly	0	0
Merck/Schering-Plough	1	1
Novartis	0	0
Ortho McNeil/Johnson & Johnson	1	0
Pfizer/Wyeth	2	0
Roche	0	0
Sanofi	0	0

It appears that industry has all but abandoned antibiotic R&D, (Table 2.1, Table 2.2). In 2009 a report identified that only five of the major pharmaceutical companies had active antibiotic discovery programs which included Pfizer, AstraZeneca, GlaxoSmithKline, Novartis, and Merck<sup>20</sup>. However, recently Pfizer and AstraZeneca have significantly reduced and/or eliminated antibiotic R&D<sup>21</sup>. At one

point AstraZeneca, Bayer, GlaxoSmithKline, Lilly, Merck, Ortho McNeil/Johnson & Johnson, Pfizer, Roche, Sanofi Aventis, Schering-Plough, and Wyeth were the international leaders in anti-infective drug discovery and development, but in recent years there have only been 3 new compounds in advanced clinical trials from these companies and a small handful in phase II or III clinical trials, as illustrated in Table 2.2<sup>21</sup>. This can mainly be attributed to the previously mentioned lack of investment in this type of research at these companies<sup>13</sup> due to the limited potential market return of antibiotics.

## THE MAIN PLAYERS IN ANTIBACTERIALS

### ASTRAZENECA

AstraZeneca (AZ) (Figure 2.4) currently markets six antibiotics<sup>L</sup>. Recently, they have been the leader of the big pharma companies in antibiotic research and development with their introduction of two antibiotics into late stage trials (Table 2). They discovered ceftaroline fosamil; an antibiotic approved for the market in 2010<sup>21</sup>. AstraZeneca markets ceftaroline fosamil as Zinforo®, in Europe, and Teflaro®, in United States markets. It is used to combat community acquired pneumonia and complicated skin and soft tissue infections (cSSI)<sup>16,22</sup>. The most well-known cSSI is the methicillin-resistant *Staphylococcus aureus* (MRSA) infection<sup>4</sup>.



**Figure 2.4.** AstraZeneca logo. Adapted from: <http://www.astrazeneca.com/Home>

Although AZ has entered the most antibiotics into phase II/III clinical trials and is one of the few companies to have active antibiotic discovery programs, in a 2013 report AstraZeneca stated their plan to decrease their antibiotic research and development programs<sup>23</sup>. They mentioned an increased focus in three categories: respiratory, inflammation and autoimmunity; cardiovascular and metabolic disease; and oncology<sup>23</sup>. At the same time they were to strive to “continue to be active in Infection & Vaccines and in Neuroscience, though our investments will be more opportunity-driven”<sup>23</sup>.

Not surprisingly, the three categories of main focus are lucrative therapeutic areas. AstraZeneca has been successful at synthesizing new antibiotics, but even they intend to slow antibiotic R&D as they are still a business with a goal of making money, as stated by their chief executive officer, Pascal Soriot:

“Our vision is clear – to be a global biopharmaceutical company with a focused portfolio in core therapy areas, underpinned by distinctive science and a growing late-stage pipeline, with sound financials offering attractive returns for investors.”<sup>23</sup>.

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<sup>L</sup> AstraZeneca currently market six antibiotics which include: Cubicin®, FluMist®, Merrem/Meronem®, Synagis®, and Zinforo®<sup>22</sup>.

The core therapies in which they invest their money are ones that have a promising financial return, such as oncology. Oncological drugs have an estimated value three times higher than antibiotics, while musculo-skeletal drugs have upwards of ten times the value<sup>14</sup>. From a business standpoint, this is logical as their narrowed focus should bring in better revenue for stock holders due to the development of medicines with high consumer costs.

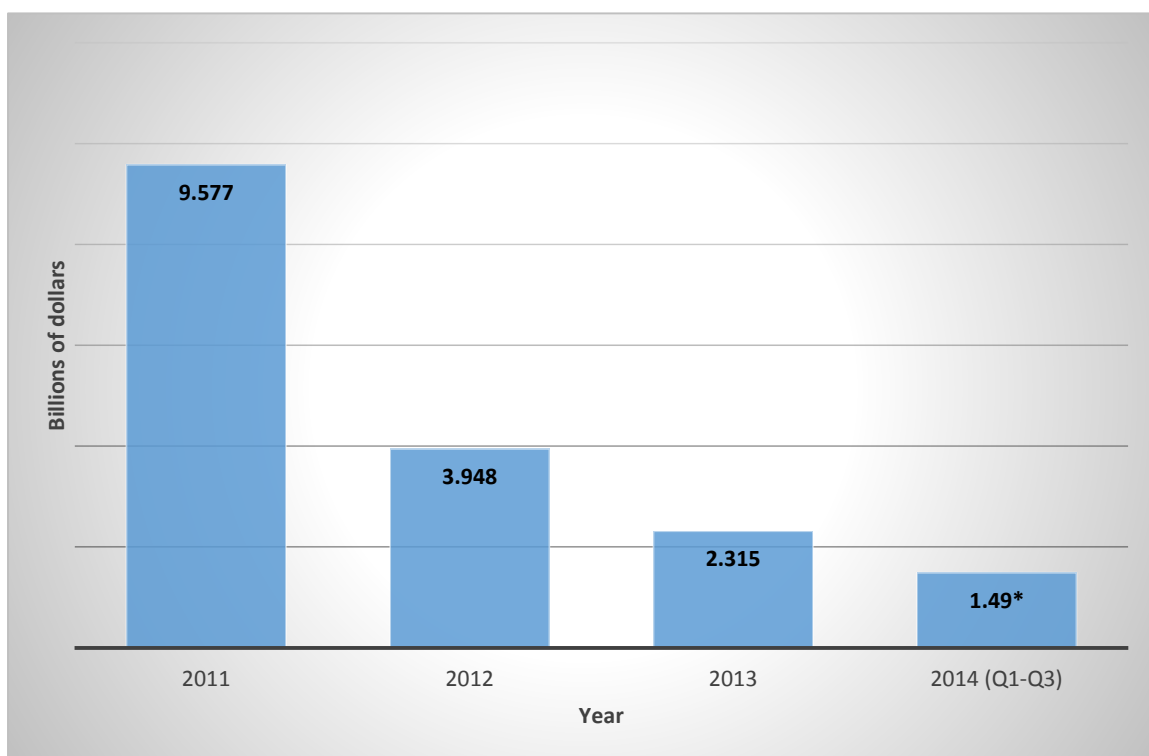
## **PFIZER**

Additionally, Pfizer (Figure 2.5), the world's largest pharmaceutical company that has historically been responsible for discovering many antibiotics, including Zithromax®, has all but abandoned its antibiotic research and development enterprise. In February of 2011, Pfizer announced the reduction in antibiotic R&D spending for 2012, thousands of lay-offs for research and development personnel, and a transition to China that would significantly slow antibiotic research and development<sup>24</sup>. Anti-infective research was to be carried out in Shanghai, China, while shutting down anti-infective research at labs in Groton, Connecticut, and Sandwich, UK.



**Figure 2.5.** Pfizer logo. Adapted from: <http://www.pfizer.com/>

The 2011 moves were designed to make up for the profit loss that the company received when it lost its patent protection for its biggest selling drug, Lipitor®<sup>24</sup>. As is common with many blockbuster drugs, Lipitor® treats a chronic condition, in this case high cholesterol. At its highest point, Lipitor® brought in \$13 billion in one year. In 2012, the patent for Lipitor® expired causing it to no longer have market exclusivity. This resulted in a significant loss of profit for the drug, decreasing sales by a staggering 59% from \$9.577 billion in 2011 to \$3.948 billion in 2012<sup>15</sup>. There was an additional 41% decrease from 2012 to 2013 with Lipitor bringing in \$2.315 billion in 2013 (Figure 2.6)<sup>25,26</sup>.



**Figure 2.6.** Lipitor® sales from 2011 to 2014. 2011 was the last year that Lipitor was under United States patent protection. \*2014 results are from the first three quarters as fourth quarter results were not yet available. Adapted from: (15), (25), and (26).

Although Pfizer halted its antibiotic R&D, they continue to sell their previously marketed antibiotics, some of which include Zosyn®, Zithromax®, Tygacil®, Sulperazon®, Dalacin®, and Unasyn®. In 2012 these antibiotics brought in a meager 3.86% of total biopharmaceutical revenue for Pfizer. In 2013 this number dropped to 3.61%<sup>M</sup> and 3.36%<sup>N</sup> in 2014 during the first three-quarters (Table 2.3, Figure 2.7). These antibiotics are listed in Pfizer’s quarterly and annual reportings as they are antibiotics that bring in \$50 million or more per quarter; however, they still produce little revenue for the company compared to other drug classes. This is a difficult dichotomy as the companies with sufficient capital for research are the ones decreasing antibiotic R&D.

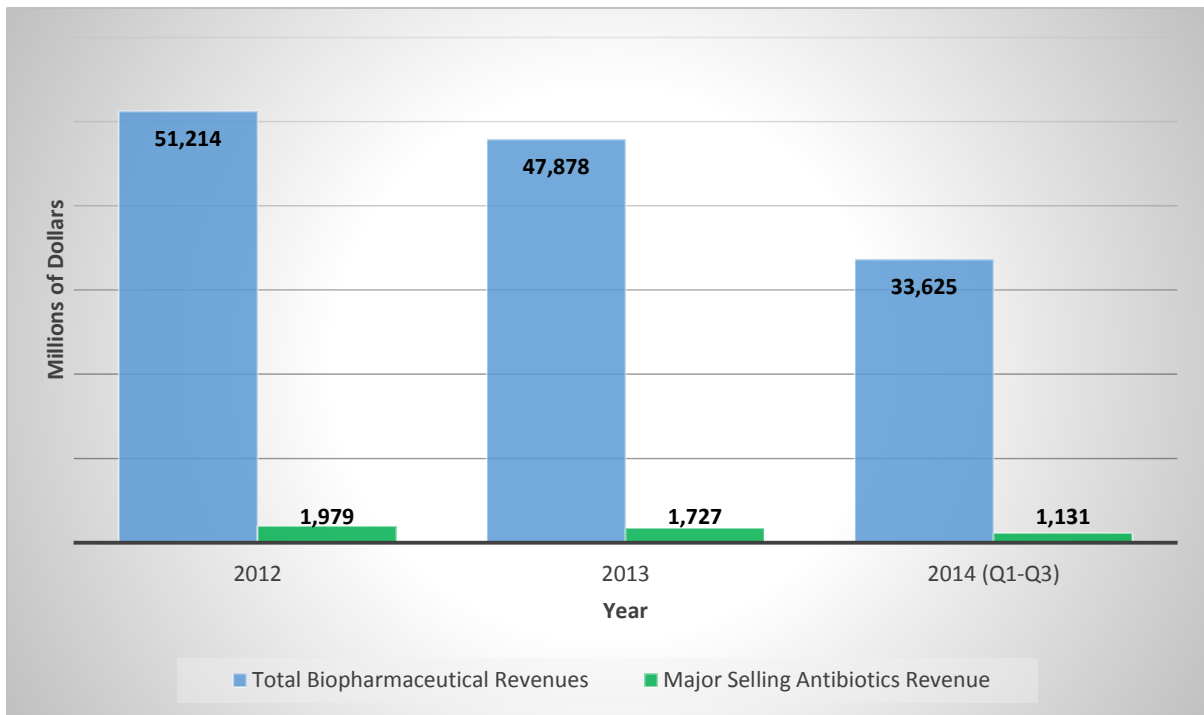
<sup>M</sup> Total Dalacin sales are not accounted for in 2013 and 2014 as only drugs with revenues at 50 million or above for each quarter.

<sup>N</sup> Fourth quarter results were not available, this number represents first three quarters of sales.



**Table 2.3.** Sales from Pfizer's major selling antibiotics from 2012 (the year after Lipitor lost its exclusivity) to 2014. Dollar values represent millions of dollars. 'Fourth quarter results were not yet available, these results are from Q1, Q2, and Q3. \*Total Dalacin sales are not accounted for as only drugs with revenues at 50 million or above for each quarter are included in quarterly reportings. Adapted from: (15), (25), and (26).

	<b>2012</b>	<b>2013</b>	<b>2014 (Q1-Q3)</b>
<b>Total Biopharmaceutical revenues</b>	\$51,214	\$47,878	\$33,625
<b>Zosyn</b>	484	395	229
<b>Zithromax</b>	435	387	235
<b>Tygacil</b>	335	358	241
<b>Sulperazon</b>	262	309	270
<b>Dalacin</b>	232	50*	50*
<b>Unasyn</b>	231	228	106
<b>Total antibiotic revenue</b>	\$1,979	\$1,727	\$1,131
<b>% of biopharmaceutical revenue</b>	3.86	3.61	3.36



**Figure 2.7** Pfizer’s total biopharmaceutical sales compared to the total sales from Pfizer’s major selling antibiotics (Zosyn®, Zithromax®, Tygacil®, Sulperazon®, Dalacin®, and Unasyn®). In 2012, antibiotics revenue was 3.86% of total biopharmaceutical sales, 2013 was %3.61 and 2014 (Quarter 1-Quarter 3) was 3.36%. Adapted from: (15), (25) and (26).

## THE PATENT PROCESS AND THE HUNGER FOR MONEY

United States patents grant protection rights of the product to the inventor “to exclude others from making, using, offering for sale, or selling the invention throughout the United States or importing the invention into the United States”<sup>27</sup>. In the United States there are three different types of patents: utility patents grant 20 years of protection from the date of grant and are reserved for the discovery or improvement of an object, composition of matter etc.; design patents grant 14 years of protection and improve the manufacture of said objects; and plant patents grant 20 years of protection and are granted to the discoverer of a new plant variety via invention or discovery and subsequent asexual reproduction<sup>27</sup>. For example, the patent that has been discussed in regards to Lipitor is a utility patent.

"To obtain a patent, an invention must be (1) novel—meaning that it has not been published more than a year before the patent application; (2) not obvious; (3) useful; and (4) adequately disclosed in the patent application to enable a scientist to practice the invention."<sup>28</sup>

The purpose of granting patents is to ensure that the developer receives proper financial compensation for their efforts. For drug discovery, this is inherently necessary due to the high cost of drug development, particularly due to clinical trials. In order for these trials to be worth the financial risk, the final drug should create enough compensation to cover the costs, and ideally, help finance future research efforts. When developing a drug, companies often start the patent process very early in the development process. This is to ensure that a drug is patented and protected when clinical trials are concluded. The average time for prosecution of a biotech patent is 4.4 years and the FDA approval process requires about 10-12 years of development<sup>29</sup>. In the United States, a patent for a drug lasts 17 years from when the patent was approved, and 20 years from when it was submitted to the patent office<sup>30</sup>, but because companies tend to start the patent process early, the patent really only provides on average 12 years of patent protection once the drug enters the market. The clinical trial and Food and Drug Administration (FDA) approval processes decrease the life of the patent, underscoring the importance of patents in new drug discovery<sup>31</sup>.

In addition, the 1984 Hatch-Waxman Act (Drug Price Competition and Patent Term Restoration Act) further increased the importance of patents, because it decreased the time required for a generic drug to enter the market once its name brand competitor lost its patent protection. The basic idea of the act works to speed up the process of entering generic drugs into the market by allowing generic equivalents to enter the market without repeating pre-clinical testing and clinical trials<sup>31-33</sup>. This helps to introduce cheaper drugs for patients sooner, but it causes the developing companies to lose revenue on their drug in a shorter time period.

A patent does not offer endless protection, as it expires after a certain amount of time allowing other companies and researchers to reproduce and sell the product. As shown in the case with Lipitor, competition from generics significantly lowers a drug's profits for a company when a patent ends and the drug loses its exclusivity. Generic drugs

generally cost significantly less than their brand name counterparts. In 2011, when Lipitor lost its exclusivity, it did not have a significantly lower out of pocket cost than its generic competitor, atorvastatin<sup>34</sup>. This was because in early 2012 only two companies were producing and marketing atorvastatin. However, later in 2012 more companies started marketing the drug, lowering the costs of the generic drug and decreasing revenue for Pfizer. Additionally, when a physician prescribes a drug, he/she does not necessarily do so with the patient's finances in mind, meaning if there is a generic option for a drug he/she may still prescribe the name brand. The decision of the drug to be distributed is often left up to the patient and the pharmacist. If a generic option is available many patients opt for it to save money. With an insurance co-pay atorvastatin could be purchased from Cigna RX1 for a 3\$ copay as compared to \$31 for Lipitor<sup>34</sup>. Additionally, the pharmacist may advocate for the generic drug as it often provides higher gross margins for them over name-brand competitors<sup>35</sup>. This being said, when a generic drug is available, the consumer will most likely purchase it which ultimately leads to decreased revenue for the discovering company.

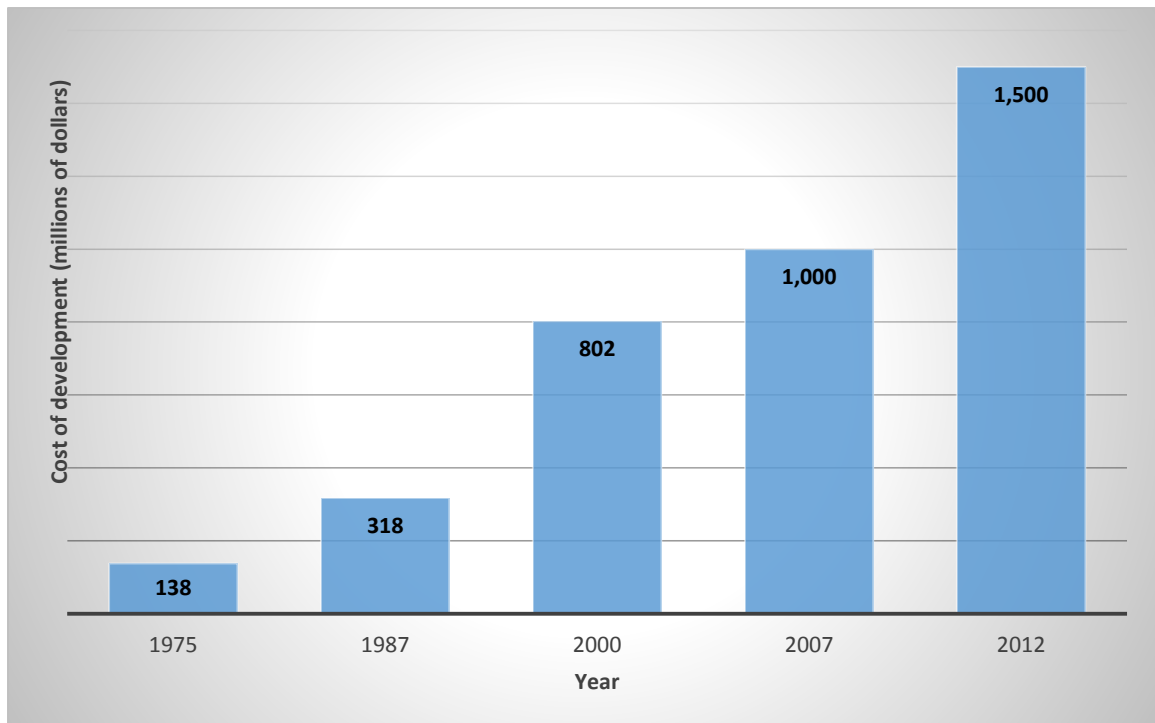
## PROMOTING INNOVATION?

Although patents pave the way for the developing company to receive profit from the product they developed, this is not the sole purpose of acquiring a patent. Research in the pharmaceutical industry relies heavily on patents<sup>35</sup>. Pharmaceutical patenting revolves around the two related influences: securing competitive market outcomes and promoting innovation<sup>35</sup>. The logic behind patents is that society agrees to give companies a temporary monopoly on the product that they develop, allowing them to market the drug at high prices, bringing in profit that can be devoted to further research, thus providing incentive to innovate<sup>36,37</sup>. To achieve a solid return when developing a new drug, business developers need to be aware of the potential market production. If business developers can predict how well their product will do in the market, then they can use this information to determine how much "innovative power" they apply to the product to ensure what they create has a promising revenue return.

A competitive market outcome relies heavily on the cost of developing a drug<sup>35</sup>. Even if a pharmaceutical creates sales, if its development is costly companies will be dissuaded from devoting time and money to it. A 2003 report estimated that a single drug costs \$802 million to develop<sup>38</sup>, which includes preclinical and clinical development. These numbers may vary based on the type of drug being developed<sup>39,0</sup>. A 2007 repeat of the 2003 study reports an increase in the cost to about \$1 billion<sup>39</sup>, while an additional study<sup>40</sup> reports it just over \$1.2 billion. More recent estimates place this value around \$1.5 billion<sup>41</sup> (Figure 2.8). With these high costs for drug development it is obvious why antibiotics do not receive much attention. If a company looks at the potential market production for an antibiotic, profit may seem out of reach, leaving companies to devote innovative power to more lucrative pharmaceuticals.

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<sup>0</sup> The estimated expected cost of developing an HIV/AIDS drug is \$479 million, while the expected cost of developing a rheumatoid arthritis drug is \$936 million (Adams2006).



**Figure 2.8.** Cost to develop a single drug in years ranging from 1975 to 2012. These costs include both research (drug discovery) and development (clinical trials and market approval) costs. Adapted from: (38), (39), (40), and (41).

Although patents are intended to promote innovation, they have caused the pharmaceutical industry to use them for profit maximization, limiting innovation towards novel antibiotics. The continuing reduction in antibiotic R&D is problematic considering the increasing threat of antibiotic resistance. After Pfizer's 2011 moves to limit, and ultimately halt, antibiotic R&D, Brad Spellberg, a member of the Infectious Diseases Society of America's (IDSA) Antimicrobial Availability task force made the statement that "the only remedy...[is] to create a new economic environment for antibiotic drugs that [restores] the financial incentives for R&D."<sup>24</sup> The question now is, is this being done, and if not, what other options are available to combat the lack of antibiotic R&D?

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## **CHAPTER 3-What is Being-Done to Address the Problem?**

Recently Big pharma has decreased its investment in antibiotic research and development and limited their spending on clinical trials. A study on the 16,055 antibiotics that have gone into clinical trials in the United States from 2000-2013 (1,235 per year) found that the majority of antimicrobial studies in the United States are funded by non-profit organizations (60%), followed by industry (30%) and the federal government (10%)<sup>1</sup>.

The decreased investment from big pharma in antibiotic research and development is a problem that if not addressed could further exacerbate the antibiotic pipeline problem and antibiotic resistance. Fortunately, there are many sectors that are devoted to addressing this almost insurmountable problem; specifically: non-profit, commercial industry other than traditional big pharma, government, and academia.

In the commercial sector, small biotech companies have increased their antibiotic R&D. This provides big pharma the opportunity to support clinical trials at a decreased cost. In the non-profit sector, the Infectious Diseases Society of America (IDSA) has started the 10x20' initiative devoted to developing new systemic antibiotics and the Bill and Melinda Gates Family Foundation is devoted to combatting the global TB crisis. In the government sector the National Institutes of Health (NIH) devotes significant sums of money on supporting antibiotic research and clinical trials; the Food and Drug Administration (FDA) advocates for improved non-inferiority trials; and the Centers for Disease Control (CDC) created the Tuberculosis Trials Consortium (TBTC) to increase clinical trials for TB. In the academic sector, the Tufts Center for the Study of Drug Development (CSDD) is devoted to assessing current drug development and finding ways to alleviate financial burdens of the process. There are several university labs such as UNC-Chapel Hill and Northeastern University devoted to developing and studying antibiotics.

## NON-PROFIT ORGANIZATIONS

### 10 x '20' INITIATIVE

The Infectious Diseases Society of America (IDSA) set a goal of creating a global antibiotic R&D enterprise to combat the antibiotic pipeline problem<sup>2</sup>. In an attempt to achieve this, the IDSA has created a global collaboration, endorsed by the American Academy of Pediatrics, American Gastroenterological Association, the Trust for America's Health, Society for Healthcare Epidemiology of America, Pediatric Infectious Disease Society, Michigan Antibiotic Resistance Reduction Coalition, National Foundation for Infectious Diseases, and the European Society of Clinical Microbiology and Infectious Diseases.



**Figure 3.1.** IDSA 10 x 20' initiative logo. Adapted from: (2)

The goal of the enterprise is to create 10 new safe and efficacious, marketable, systemic<sup>P</sup> antibiotics by 2020, otherwise known as the 10 x '20 initiative (Figure 3.1)<sup>2</sup>. To be successful this initiative requires many feats, but one of the main ideas is to include the leaders in global political, scientific, industry, economic, intellectual property, policy, medical and philanthropic fields<sup>2</sup>. However, since the IDSA's report in 2009, only two new systemic antibiotic agents have been approved, which are telavancin (Theravance), and ceftaroline fosamil (AstraZeneca). Of these only ceftaroline fosamil<sup>Q</sup> has potential to be a sought after 10 x '20 drug<sup>5</sup>. Although the initiative is a worthy cause, it is very unlikely that this goal will be met by 2020.

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<sup>P</sup> Systemic means that the antibiotic affects the entire body when administered. It follows the ADME path or Absorption, Distribution, Metabolism, and Excretion. The drug first is absorbed into the bloodstream, distributed throughout the body, metabolized and broken down, then metabolites are excreted. ADME screening is used to analyze and develop doses for the drug<sup>3</sup>.

<sup>Q</sup> Ceftaroline fosamil has shown efficacy against *Staphylococcus aureus* (the bacteria responsible for MRSA infection) and *Enterobacteriaceae*<sup>5</sup>, two of the pathogens that effectively "ESKAPE" the effects of current antibiotics<sup>4</sup>. The ESKAPE pathogens are *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species<sup>4</sup>.

Despite the slow process of the 10 x '20 initiative, there has been some recent and upcoming success in local antibiotics, specifically for TB. In addition to the two new systemic antibiotics previously mentioned, four additional antibiotics, antofloxacin (Shanghai Institute of Materia Medica), besifloxacin (Bausch & Lomb), fidaxomicin (Optimer Pharmaceuticals), and bedaquiline (Janssen Pharmaceuticals), have been approved since 2009<sup>5</sup>. In December of 2012, the partnership between Tibotec, part of Janssen Pharmaceuticals, and the Global Alliance for TB drug development led to the approval of bedaquiline, a first in class antibiotic and the first new TB treatment in over 40 years<sup>5</sup>. There are currently six other potential TB drugs in clinical development, including delamanid (phase-III, Otsuka Pharmaceutical), perchlozone<sup>R</sup> (complete phase-II/III, JSC Pharmasyntez), SQ109 (phase-III, Sequella), PA-824 (phase-II, TB Alliance), sutezolid (phase-II, Pfizer) and posizolid (phase-II, AstraZeneca), as well as several others still in pre-clinical development<sup>6-9</sup>.

## **THE BILL AND MELINDA GATES FAMILY FOUNDATION**

The Bill and Melinda Gates Family foundation works with partner organizations to tackle some of the world's most critical problems including poverty, malaria, HIV, and TB. Increased funding is needed for proper TB research and development. One of the main goals of the Bill and Melinda Gates Family Foundation is to raise these required funds. The foundation works with the Global Fund to Fight AIDS and the Tuberculosis and Malaria UNITAID in order to reduce the cost of innovative technologies<sup>10</sup>. The foundation's efforts in the TB epidemic range over many levels. The current TB regimen involves several drugs<sup>S</sup>, and, as mentioned, there are strains of bacteria resistant to these drugs, propelling the need for novel TB antibiotics. In addition, TB cannot be effectively treated with a single drug, causing the development of new TB drugs to require multiple clinical trials for a completely new treatment. The significant time and cost of clinical trials could cause this process to take decades<sup>10</sup>, and the foundation is attempting to help accelerate this process.

“To address this obstacle, we have joined with partners to create the Critical Path to TB Drug Regimens (CPTR) initiative, which brings together leading international

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<sup>R</sup> Perchlozone is currently approved in a limited market in Russia.

<sup>S</sup> Isoniazid, Rifampin, Pyrazinamide, and Ethambutol.

pharmaceutical companies, public health experts, nongovernmental organizations, and U.S. and other regulatory authorities to expedite testing of promising TB drug candidates in combination and to identify new regulatory pathways and other means of accelerating the drug development process”<sup>10</sup>.

The most direct way of preventing the spread of TB is the development of a preventative vaccine. The current BCG vaccine provides limited protection for newborns, and no protection for pulmonary TB in adults, which is the form of TB that causes the most deaths. A new vaccine, even if partially effective, could help decrease TB incidence, according to some projections, up to 52% by 2050<sup>10</sup>. The first candidate vaccine made it through phase III clinical trials, but due to its inability to protect infants, it was not marketed. Similar to the development of novel TB drugs for treatment, the development of a vaccine can take many years; therefore the foundation also aims to discover innovative and accelerated approaches to vaccine development<sup>10</sup>.

## COMMERCIAL INDUSTRY

### SMALL BUSINESS INVESTMENT-DISCUVA



**Figure 3.2.** Discuva. Adapted from: (12)

The current trend in antibiotic research and development has shifted from large pharmaceutical companies towards smaller organizations. Specifically, Discuva (Figure 3.2), a small biotech company based in Cambridge, United Kingdom, is devoted to the idea of “one bug, one drug” antibiotics. In the past, antibiotics have been developed to treat many bacterial infections. One example is azithromycin, or commonly Zithromax®, which treats multiple infections including skin infections, ear infections, and sexually transmitted diseases<sup>11</sup>. However, due to increased antibiotic resistance these once panaceas no longer have the ability to stop all of the bacterial infections they were intended for and have become ineffective against Gram-negative bacterial infections. There is currently a significant lack of development in new classes of antibiotics which is the exact problem that Discuva is addressing. By attempting to develop novel classes of antibiotics, or “one drug for one bug”, they eliminate significant risks of antibiotic resistance present in multi-target antibiotics<sup>12</sup>.

Discuva employs SATI<sup>N</sup> (Selective Antibiotic Target Identification) technology to identify specific molecular targets and genes that have the potential to cause antibiotic resistance and reduce the antibiotic’s efficacy<sup>13</sup>. In essence researchers can see what the compound is doing inside of the bacteria, a process that cannot be obtained with traditional biochemical techniques<sup>T</sup>. The use of this technology ensures that only compounds that

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<sup>T</sup> Traditional biochemical techniques for identifying drug candidates are serendipity, screening, or design. Serendipity is when the target is incubated with the potential drug and it is observed if the target lives or dies (such as a bacteria) or if they affected improves (mouse model affected with the disease). Screening involves taking a compound that is known to have beneficial effects is modified slightly to see if the new compound has different effects. Design involves designing drugs to fit into the active site of an enzyme that is identified to be a possible drug target<sup>14</sup>.

have the potential to make it through clinical trials are taken further into the drug development process. This can save significant sums of money by eliminating unnecessary clinical trials in addition to decreasing the risk of the development of antibiotic resistance<sup>15</sup>.

## **THE INDUSTRY DICHOTOMY**

Although small biotech companies, like Discuva, are adopting more antibiotic R&D, they often do not have the financial means for pushing drugs through clinical trials. The dichotomy for a proper business model stems from big pharma's investment in lucrative therapeutic categories and the lack of capital available for small companies further down the line of antibiotic research and development<sup>16</sup>. Small biotech companies have the means to perform the early stage of drug development, however they do not have the financial capital to drive potential drugs through phase II/III trials<sup>17</sup>.

In the past, and today, small biotech companies have needed support from big pharma companies to push their drugs through phase II/III trials. For example, the Boulder, Colorado based biotech company Array Biopharma, researches several anticancer drugs. Oncology is a lucrative therapeutic area, but the company still requires financial support from outside sponsors. Array has several anticancer drugs in clinical trials. The clinical trials for binimetnib, a potential thyroid cancer drug, are supported by Novartis. The clinical trials for selumetinib, a potential drug for the treatment of several types of cancer, are supported by AstraZeneca<sup>18</sup>.

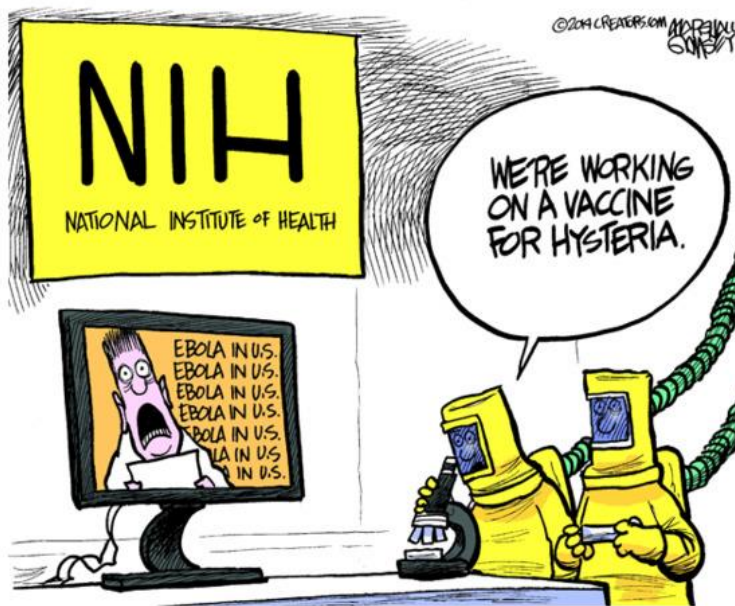
Although many small biotech companies do not have the means to support phase II/III trials, this may provide big pharma companies with an avenue to make profit at a decreased cost. The small biotech companies can synthesize potential drug candidates and relieve monetary costs of pre-clinical drug development. This provides big pharma with the option of supporting clinical trials for the proposed drug without having to pay for the discovery process. Additionally, big pharma has the option of choosing the best drug candidate. For example, say there are four small biotech companies that have identified potential TB drugs. The supporter of clinical trials has the power to choose which company to support for clinical trials by choosing the most promising drug candidate which



eliminates the supporter's cost of early drug-development, decreases the risk of failed clinical trials, and offers the potential of an investment return.

## GOVERNMENT

### NATIONAL INSTITUTES OF HEALTH (NIH)



**Figure 3.3.** A comic generalization of the NIH's involvement in national health.  
<http://theweek.com/cartoons/index/270411/editorial-cartoon-nih-hysteria-vaccine>

Of the 16,055 antimicrobial clinical trials in the U.S. from 2000-2013, 6.5% of them were funded by the National Institutes of Health (NIH)<sup>1</sup>. The NIH is made up of 27 institutes and centers ranging anywhere from the National Cancer Institute (NCI) to the National Institute on Aging (NIA)<sup>19</sup>. The institute's storied involvement with national health can

partly be attributed to its long standing existence. The NIH informally started in 1798 when John Adams helped establish the Marine Hospital Service. "NIH's mission is to seek fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to enhance health, lengthen life, and reduce illness and disability"<sup>19</sup> (Figure 3.3).

The NIH is working to increase collaboration for the improvement of disease in general. The NIH recently opened the NIH Clinical Center, a premier research hospital, to non-government researchers through 3-year renewable research grants up to \$500,000 per year. This allows researchers to collaborate with NIH investigators to work towards

translating laboratory discoveries to improved diagnosis, prevention, and treatment of disease<sup>19</sup>.

Almost 80% of the NIH's funding is awarded through competitive grants. The institute invests almost \$30.1 billion to American medical research annually. In 2013 they devoted \$240 million to tuberculosis research, \$279 million in 2014, and they project to spend \$279 million in 2015. Additionally, research on TB vaccinations was \$26 million in 2013, \$31 million in 2014, and they project to spend \$31 million in 2015<sup>20</sup>. They devoted over \$5 billion to infectious diseases<sup>U</sup> research in 2014. The largest spending area by far was clinical research with \$10.6 billion devoted to the area (Table 3.1). The increase across all mentioned areas is encouraging in regards to addressing antibiotic funding.

**Table 3.1.** NIH Categorical spending in different research areas from 2010-2015. Monetary values are in millions of dollars. Adapted from: (20)

<b>Research/Disease Areas</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>2015 (Estimated)</b>
<b>Clinical Research</b>	\$10,503	\$10,951	\$10,604	\$11,087	\$11,132
<b>Clinical Trials</b>	\$3,093	\$3,208	\$3,155	\$3,221	\$3,233
<b>Infectious Diseases</b>	\$3,883	\$3,867	\$4,887	\$5,002	\$5,015
<b>Tuberculosis</b>	\$209	\$218	\$240	\$279	\$279
<b>Tuberculosis Vaccine</b>	\$17	\$21	\$26	\$31	\$31

<sup>U</sup> Infectious diseases include any disease caused by a microorganism such as a bacteria, fungi, or parasite.

## **OTHER GOVERNMENT AGENICIES**

Less than 1% of the 16,055 antimicrobial studies were funded by government agencies besides the NIH. One important agency is the Food and Drug administration (FDA) and another that specifically supports clinical trials is the Centers for Disease Control and Prevention<sup>25</sup>.

## **FOOD AND DRUG ADMINISTRATION**

The FDA is the government agency that ultimately approves drugs to enter the market. The FDA requires that trials include primary safety and efficacy endpoints as clinical studies “form the basis for FDA’s finding that a [drug or device] is safe and effective for its intended use”<sup>21</sup>. In addition, it provides guidance and regulations for clinical trials. Specifically, in 2010, the FDA proposed further guidelines for antibacterial non-inferiority (NI) trials to further support drug approval. Non-inferiority trials (as opposed to superiority trials, which use placebos to observe efficacy of the new treatment) are intended to show that the new treatment is not worse than the control treatment (the previously used treatment for the indicated condition)<sup>22</sup>. NI trials can be used in cases where there is resistance, as it will demonstrate that the new treatment has better efficacy over drug-resistant bacteria than the standard treatment. One of the reasons that the FDA is addressing NI trials is because of the ethical issues involved in superiority trials. As mentioned superiority trials involve the use of placebo in comparison to the new treatment. When a person signs up for a clinical trial they expect to receive the best therapeutic method possible<sup>23</sup>. But, in superiority trials some of the participants receive a placebo, thus they receive no treatment. In a trial for a cholesterol drug this may not be as much of an issue as the affliction is not immediately life-threatening. However, if a cancer patient enters a clinical trial they expect to receive at least some form of treatment. In a life-threatening disease this is an issue as a lack of treatment could be detrimental to the patient’s health and life.

The FDA suggests that the sponsor of the trial uses an active-controlled NI trial design to consider the potential treatment effect of the control treatment<sup>24</sup>. This can be achieved from analyzing previously conducted trials of the control treatment. This is to ensure there is proper control and efficacy endpoints from previous trials to determine the

treatment effect size (which measures the magnitude or size of an effect, or the effect of the treatment) for the newly proposed NI trial. Additionally, the FDA suggests that the sponsors should re-evaluate current and ongoing NI trials on the scientific basis for the treatment effect size of the control. After evaluation, the approval application should be amended to reflect these findings. However, if the reevaluation does not provide necessary scientific basis then FDA commitments may no longer be valid<sup>24</sup>.

### **CENTER FOR DISEASES CONTROL TUBERCULOSIS TRIALS CONSORTIUM**

The CDC's slogan "CDC 24/7" (Figure 3.4) tells people that "CDC is the nation's health protection agency, working 24/7 to protect America from health and safety threats, both foreign and domestic. CDC increases the health security of our nation"<sup>25</sup>. The CDC aims to protect the nation from health threats, from simple measures like the prevention of a common cold, to the tuberculosis



**Figure 3.4.** Centers for Disease Control and Protection's (CDC) slogan, CDC 24/7. Adapted from: (25)

epidemic, and the recent Ebola outbreak in West Africa<sup>25</sup>. Specifically, in 1960 the U.S. Public Health Services transferred its TB control and research program to the CDC. The 1980's saw a decline in TB, and consequently a decline in funding for TB. In response, the CDC Tuberculosis Trials Consortium (TBTC) was officially created in 1997 to increase clinical trials for TB. In the current decade (2010-2020) the TBTC aims to move study sites from the United States to international locations to address the global prevalence of the disease<sup>26</sup>. The TBTC has enrolled more than 12,000 patients and volunteers in the past 15 years, and has an annual budget of \$11 million annually. The TBTC is currently conducting a trial for an ultra-short treatment of latent TB, and has begun the first clinical trial for patients with MDR-TB. According to the CDC "the late pipeline of new anti-TB drug candidates is the most promising in 40 years, and advances in TB clinical trials science have fostered the progress of these agents"<sup>26</sup>.

## ACADEMIA

According to the CDC “developing new TB treatment and prevention strategies depends upon collaboration among academic, private sector and government researchers and non-governmental organizations”<sup>26</sup>. This need for collaboration is not limited to TB and is relevant for all antibiotic R&D. Specifically, universities can play a role in antibiotic R&D.

### TUFTS CENTER FOR THE STUDY OF DRUG DEVELOPMENT

The Tufts Center for the Study of Drug Development (CSDD) is an independent, academic, non-profit research group at Tufts University in Boston, Massachusetts<sup>27</sup>. Their mission is to “develop strategic information to help drug developers, regulators, and policy makers improve the quality and efficiency of pharmaceutical and biopharmaceutical development, review, and utilization”<sup>27</sup>. In 2013 they held a Senior Leadership Roundtable (Figure 3.5) to discuss the adoption and impact of adaptive clinical trial<sup>v</sup> design<sup>28</sup>. Based on the results of the roundtable, simple adaptive designs are

currently being used in 20% of clinical trials and this number is expected to continue to increase<sup>28</sup>. According to the Tufts CSDD the main prospect in aiding antibiotic development efforts is for developers to cut extraneous costs. Clinical trials are the most cost intensive portion of developing drugs. They run hundreds of millions of dollars<sup>29</sup> making clinical trials a strong area of interest in decreasing costs.



**Figure 3.5.** The Tufts CSDD Senior Leadership Roundtable brief cover art. Adapted from: (28).

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<sup>v</sup> Adaptive clinical trials are preplanned adaptations — generated through the use of trial simulations and scenario planning — of one or more specified clinical trial design elements that are modified and adjusted while the trial is underway based on an analysis of interim data<sup>29</sup>.

Only about one in one-thousand compounds discovered in pre-clinical drug-discovery will make it all the way through the FDA approval process and enter the market as a drug<sup>30</sup>. Drugs that fail to make it through clinical trials cause the cost of clinical trials and drug development to increase significantly. In order to alleviate some financial burden, studies involving compounds that will not make it through clinical trials can be terminated early. The Tufts CSDD estimates that early termination of studies due to uselessness and sample size re-estimation<sup>W</sup> could save sponsor organizations \$100-\$200 million annually<sup>28</sup>. One company present at the roundtable reported saving \$70 million by implementing simple adaptive clinical trial designs<sup>28</sup>.

Additionally, using adaptive clinical trial designs for phase II/III dose response assessments (these serve to assess harmful side effects of treatment in response to dose) are expected to improve late stage success rates. Regulatory agencies see this as the most promising benefit from the adaptive clinical trial design.

Industry has devoted a significant amount of attention to the overall improvement of quality and efficiency of clinical trials, which coupled with adaptive clinical trial designs, shows promise in improving overall R&D<sup>28</sup>.

## **UNIVERSITY RESEARCH**

### **NORTHEASTERN UNIVERSITY: ANTIMICROBIAL DISCOVERY CENTER**

The Antimicrobial Discovery Center (ADC), headed by Dr. Kim Lewis, is a molecular microbiology research group at Northeastern University that studies bacterial persister cells, drug discovery, unculturable microorganisms, and *Mycobacterium tuberculosis*<sup>31</sup>. Specifically, the ADC's work in regards to Mtb involves the study of the mechanism of persister bacteria. Persisters are dormant bacteria that remain in the host and are tolerant to antibiotics<sup>31</sup>. The ADC is working on the discovery of sterilizing antibiotics, which completely eradicate all of the bacteria in a bacterial infection, including persisters. On this topic specifically, they collaborate with scientists from the small biotech companies NovoBiotic and Arietis<sup>31</sup>.

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<sup>W</sup> Sample size re-estimation is adjusting the sample size in a clinical trial based on interim data to ensure that the sample size is large enough to accurately assess a drug's efficacy<sup>32</sup>.

## UNIVERSITY OF NORTH CAROLINA-CHAPEL HILL: MEDICINAL CHEMISTRY

The University of North Carolina-Chapel Hill Eshelman School of Pharmacy has a division in chemical biology and medicinal chemistry<sup>33</sup>. Specifically Dr. Harold Kohn's lab focuses on bacterial infections. Their lab discovered bycyclomycin, an antibiotic effective against Gram-negative bacteria, specifically *Escherichia coli*. Bycyclomycin acts on the rho transcription termination factor, an essential enzyme in *E. coli*. The lab is now attempting to identify the exact mechanism of bycyclomycin, and the rho enzyme in order to allow drug design to occur on a less empirical basis<sup>34</sup>.

Additionally, the Kohn lab identifies that resistance to conventional antibiotics, such as those for TB, is an unmet challenge. Therefore, they are currently pursuing research towards the identification of novel TB antibiotics. They have identified metal chelating<sup>x</sup>, pathogen specific inhibitors for *Mycobacterium tuberculosis* and have information regarding the target site for the compounds<sup>34</sup>. These type of inhibitors are currently of great interest in the search for novel TB antibiotics.

Academia is an area that could aid in antibiotic research and development efforts. The Tufts CSDD's aim is to help researchers improve efficiency in developing drugs while the ADC and Kohn's lab at UNC are devoted to laboratory research on antibiotics. Chemical industry relies on the principle "time is money". This is part of the reason that they have limited investment in antibiotic R&D. Although the patent process, rigor of industry, and prospect of monetary return aims to promote innovation, innovation is often pushed aside. However, academic laboratory research provides a resource where innovation is at the forefront and researchers are free to devote time and resources to antibiotic research development.

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<sup>x</sup> Metal chelating refers to compounds that have the ability to attach to positively charged metals, binding to the area where the metal is situated.

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## **CHAPTER 4- Synthesis of potential *Mycobacterium tuberculosis* class IIa fructose-1,6-bisphosphate aldolase inhibitors**

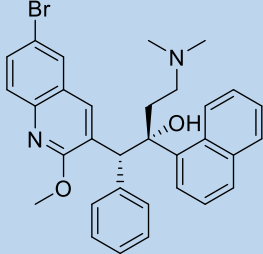
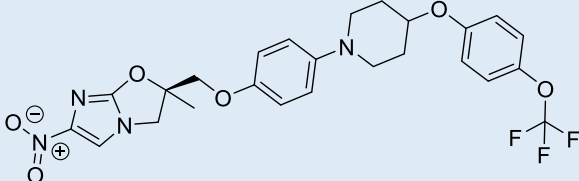
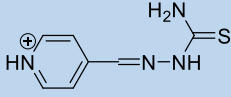
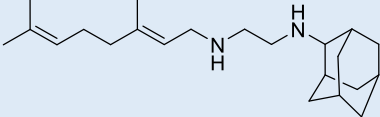
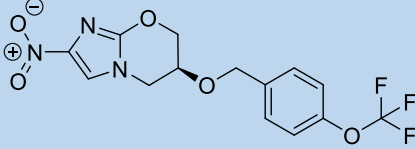
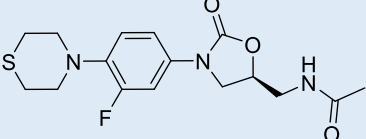
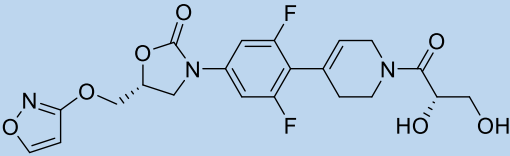
This chapter describes a project I worked on from August 2013 to May 2014 directed towards the identification of inhibitors of *Mycobacterium tuberculosis* class IIa fructose-1,6-bisphosphate aldolase (MtFBA), an essential enzyme in *Mycobacterium tuberculosis*. This enzyme serves as a potential target for novel TB therapeutics. In 2010 a collaboration was initiated between Dr. Kateri Ahrendt of Regis University, and Dr. Scott Pegan of the University of Denver (now at the University of Georgia). Later, Dr. Mary Jackson of Colorado State University joined the group. They have worked on a collaborative research project in identifying MtFBA inhibitors. Dr. Ahrendt's lab synthesizes potential inhibitors, Dr. Pegan's lab tests synthesized compounds for enzymatic activity, while Dr. Jackson's lab tests for cellular activity. This research aims to identify scaffolds that could serve as potential antibiotics to combat the TB epidemic. While working in Dr. Ahrendt's lab, I played a role in the synthesis of two sulfonamide containing compounds. Several Regis University students have been involved in the MtFBA project including; Alex Moauro, Nicholas Stephanus, Marina Pschichenko, Patrick Serrano, Pablo Cabrera, and Christian Ghincea. I would also like to acknowledge the Regis University Chemistry Department for access to laboratory facilities, equipment, chemicals and supplies, the Regis University Dayton Memorial Library for access to scientific databases and electronic journal subscriptions, the Regis University Research and Scholarship Council for financial support, and the Colorado Center for Drug Discovery for financial support.

## INTRODUCTION

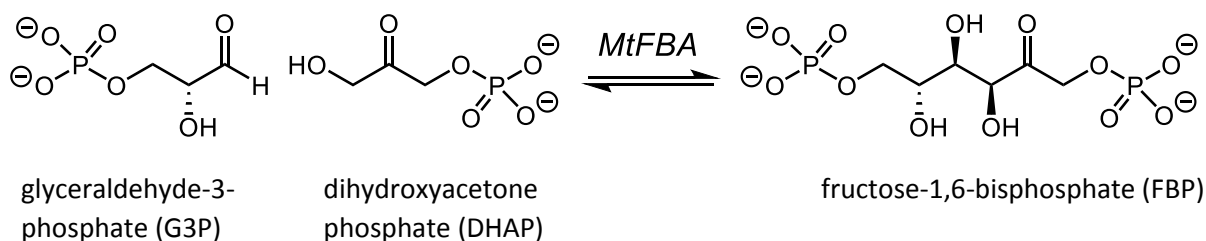
Tuberculosis is currently second to HIV as the greatest killer due to a single infectious agent<sup>1</sup>. Approximately one third of the global population is infected with latent TB<sup>2</sup> and approximately 10% of these individuals will develop the active disease in their lifetime<sup>1</sup>. The current TB chemotherapy, recommended by the World Health Organization, involves four drugs over a six month period. Isoniazid (INH), Rifampin (RIF), Pyrazinamide (PZA), and Ethambutol (EMB) are taken for two months followed by a continuation of INH and RIF for an additional four months<sup>2</sup>. The lengthy treatment leads to patient compliance issues and the development of drug resistant TB. This includes MDR-TB, resistant to at least INH and RIF, and XDR-TB resistant to INH and RIF and additional potent TB antibiotics<sup>1,3</sup>.

In December 2012, bedaquiline, a first in class antibiotic and the first new TB treatment in over 40 years, was approved by the FDA<sup>4</sup>. There are currently six other potential TB drugs in clinical development, including delamanid (phase-III), perchlozone (complete phase-II/III), SQ109 (phase-III), PA-824 (phase-II), sutezolid (phase-II) and posizolid (phase-II), as well as several others still in pre-clinical development<sup>4</sup> (Table 4.1). However, these recent TB antibiotic successes do not have the ability of eradicating the disease as they do not significantly decrease the current length of treatment, thus propelling the need for the discovery of new TB antibiotics.

**Table 4.1.** Current potential tuberculosis drug candidates and their respective clinical trial phase of development. Adapted from: (4)

Name of Drug	Structure	Phase of Development
Bedaquiline	 <p>The structure of Bedaquiline features a central chiral center bonded to a 4-bromo-2-methoxyquinoline ring, a phenyl ring, a hydroxyl group, and a dimethylaminoethyl side chain.</p>	Approved
Delaminid	 <p>The structure of Delaminid consists of a 5-nitroimidazole ring system connected via a chiral center to a piperazine ring, which is further linked to a 4-(trifluoromethoxy)phenyl group.</p>	Phase II
Perchlorone	 <p>The structure of Perchlorone is a 2-mercapto-1H-imidazole ring with a protonated nitrogen and a methylamino group at the 4-position.</p>	Complete phase II/III
SQ109	 <p>The structure of SQ109 is a long-chain polyene with a terminal bicyclic amine moiety.</p>	Phase III
PA-824	 <p>The structure of PA-824 is a 5-nitroimidazole ring system connected via a chiral center to a piperazine ring, which is further linked to a 4-(trifluoromethoxy)phenyl group.</p>	Phase II
Sutezolid	 <p>The structure of Sutezolid features a piperazine ring substituted with a thiomorpholine ring and a fluorine atom, connected to a thiazolidine ring which has an acetamido group.</p>	Phase II
Posizolid	 <p>The structure of Posizolid is a complex molecule featuring a thiazolidine ring, a piperazine ring, and a dihydroxyethyl side chain, with multiple fluorine substituents.</p>	Phase II

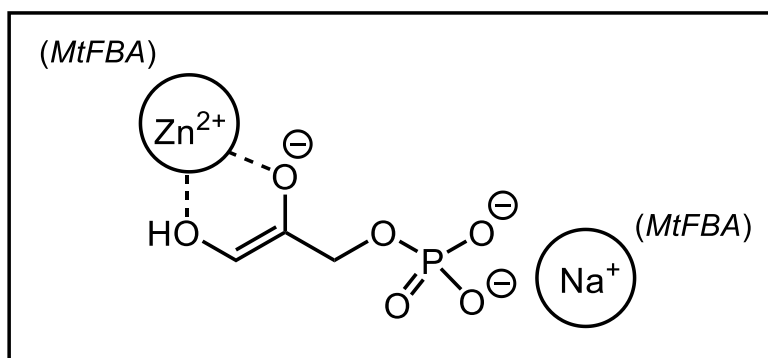
One potential TB therapeutic target is *Mycobacterium tuberculosis* class IIa fructose-1,6-bisphosphate aldolase (MtFBA). Fructose-1,6-bisphosphate aldolase (FBA) is an essential enzyme present in most organisms, including animals, plants, fungi, and bacteria. The enzyme catalyzes the reversible aldol condensation of glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP) to fructose-1,6-bisphosphate (FBP) (Figure 4.1) in glycolysis and gluconeogenesis<sup>5,6,7</sup>. Glycolysis and gluconeogenesis are the essential carbohydrate break down and build up pathways of the cell and are essential for cellular survival, thus inhibition of an essential enzyme in either pathway has the potential to kill the cell. Knockout studies<sup>8</sup> have shown that cells cannot survive without FBA.



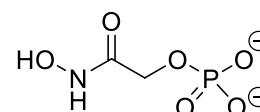
**Figure 4.1.** Reversible aldol condensation of G3P and DHAP to FBP catalyze by MtFBA. Adapted from: (5).

While FBA is a ubiquitous enzyme, bacterial FBA is distinctively different than human FBA in both protein structure and mechanism of catalysis<sup>5,6,9,10</sup> providing the opportunity for selective inhibition of bacterial FBA. Class I FBAs, present in humans and mammals, catalyze the aldol condensation using a lysine residue and Schiff base formation, whereas class II FBAs, present in bacteria, catalyze the reaction by stabilization of the hydroxyenolate intermediate with Zinc (II) and sodium ions (Figure 4.2)<sup>5,6</sup>. Because of the distinct structure and mechanism of catalysis, class II FBAs have the potential to serve as antimicrobial targets as they can potentially be selectively inhibited over human class I FBA.

**Figure 4.2.** MtFBA and other class II FBAs use Zinc (II) and sodium ions to stabilize the enolate intermediate. Adapted from: (5).

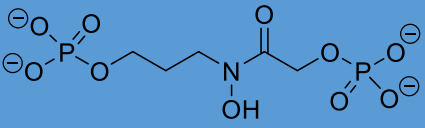
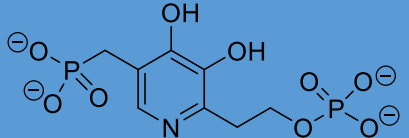
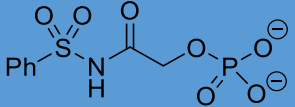
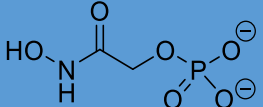
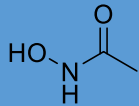


Previously reported phosphate containing FBA inhibitors have shown activity in bacterial cultures (Table 4.1). The most extensively studied MtFBA inhibitor is phosphoglycolhydroxamic acid (PGH, Figure 4.3) which mimics the FBA enolate substrate, DHAP (Figure 4.1). However, the hydroxamic acid poses potential hazardous side effects<sup>9</sup> and due to the similarity in structure it shares with DHAP, it lacks selectivity for class II over class I FBAs. Furthermore, PGH lacks the necessary physiological properties for drug development<sup>5</sup>. Specifically, the highly charged phosphate group prevents cell permeability and is easily hydrolyzed within the cell. However, removal of the phosphate group leads to a loss of enzyme inhibition (Table 4.2). As such, novel MtFBA inhibitors may be able to overcome some of these limitations.



**Figure 4.3.** PGH

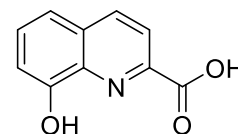
**Table 4.2.** IC<sub>50</sub> of several phosphate containing inhibitors. The IC<sub>50</sub> measures the concentration of a drug where the enzyme's activity is cut in half. \*demonstrates inactivity of compounds without the phosphate group.

Compound	Bacteria	IC <sub>50</sub>	Citation
	<i>Mtb</i>	13nM	11. Daher 2010
	<i>Giardia</i>	15μM	12. Mariano 2011
	<i>E. coli</i>	100μM	13. Blonski 2005
	<i>Mtb</i>	2nM	14. Lewis 1973
		Inactive at 1mM*	

To identify potential MtFBA inhibitors, we are using a fragment based approach. In this approach, a range of small Zinc (II) chelating molecules are screened at high concentration to assess the extent of MtFBA inhibition. The initial compounds are readily available, either through commercial search or straightforward synthesis. Compounds with highly charged functional groups, such as phosphates, are avoided to facilitate cell permeability and bioavailability. The data from initial screens is then used to design subsequent target compounds<sup>15</sup>.

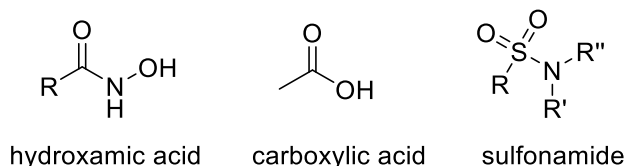


Before I joined the research project, using the fragment based approach, the group identified commercially available 8-hydroxyquinoline-2-carboxylic acid (HCA, Figure 4.4) as a 10  $\mu$ M inhibitor of MtFBA<sup>Y</sup>.



**Figure 4.4.** HCA

A significant portion of the fragment based approach involves identifying metal chelating groups. The most common metal chelating groups are hydroxamic acids, carboxylic acids, and sulfonamides<sup>15</sup> (Figure 4.5). As mentioned, charged phosphate groups hinder the cell permeability of the compound. Thus, the ideal metal chelating inhibitor of class II FBAs is one that exists in its neutral form to an appreciable extent outside of the cell, allowing entry and permeability of the cell membrane. Once in the cell, the compound should be able to adopt a negative charged state, facilitating enzyme interaction.



**Figure 4.5.** Common metal chelating functional groups

We decided to synthesize sulfonamide containing compounds because of their ability to chelate Zinc (II) ions when charged. Sulfonamides have an acid dissociation constant<sup>Z</sup> that may allow the compound to serve as a negatively charged Zinc (II) chelator at physiological pH<sup>16,17</sup>. Importantly an equilibrium exists between the neutral and charged state; the acid dissociation constant is a measure of this equilibrium. The sulfonamide acid dissociation constant is at a value where the compound has a significant neutral population outside of the cell allowing cellular entry. Then once inside the cell, the sulfonamide proton (H<sup>+</sup>) can be removed creating a negatively charged nitrogen that may serve to chelate with Zinc (II). Compounds with

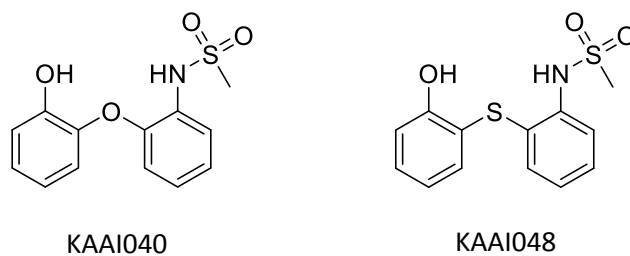
<sup>Y</sup> A 10 $\mu$ M inhibitor indicates that the IC<sub>50</sub> (half maximal inhibitory concentration) of the compound is when the compound is present in a 10 $\mu$ M concentration. The IC<sub>50</sub> is the concentration of inhibitor that decreases and enzyme's maximum velocity by one half.

<sup>Z</sup> The acid dissociation constant of an acid. The lower the number the more easily the compound releases a hydrogen atom and becomes negatively charged.

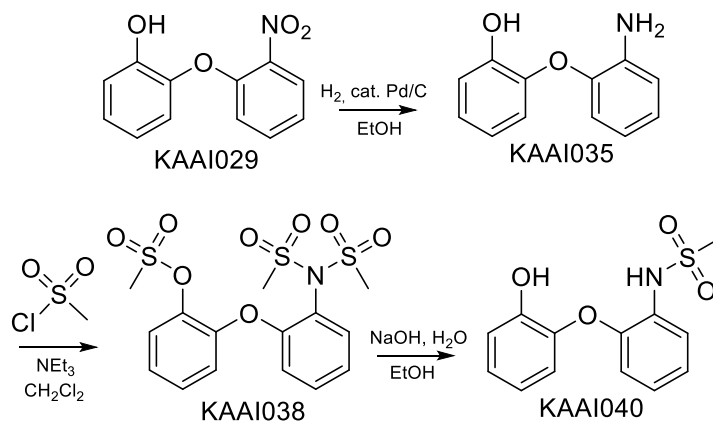
sulfonamides have shown increased extraction selectivity for Zinc (II) ions over other metal ions in solution<sup>16</sup>. Additionally, the most widely used probes for cellular Zinc (II) are aryl-sulfonamides of 8-aminoquinoline<sup>18</sup> demonstrating the ability of these compounds to permeate the cellular membrane. This led us to synthesize aryl-sulfonamide compounds that have the potential to chelate Zinc (II) and have cellular membrane transport.

## RESULTS

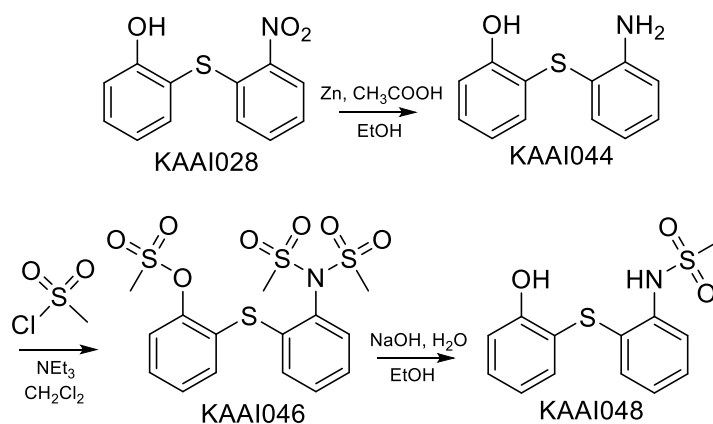
We successfully synthesized two bridged biaryl-sulfonamide compounds (KAAI040, KAAI048) (Figures 4.6, 4.7) via multi-step syntheses (Schemes 4.1, 4.2). Each step in the overall synthesis was verified with proton nuclear magnetic resonance (NMR) and thin layer chromatography (TLC). (The detailed experimental procedure is addressed in the later experimental section.) The final target compounds and intermediates in the multi-step syntheses were sent to Dr. Pegan at the University of Denver to assess MtFBA inhibition. The compounds were tested in a fluorescence assay. The inhibition results are presented in Table 4.3.



**Figure 4.6.** Final target compounds for MtFBA inhibition, KAAI040 and KAAI048

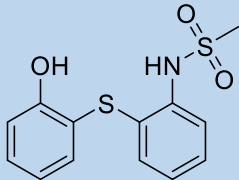
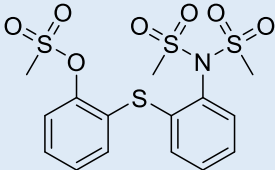
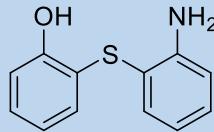
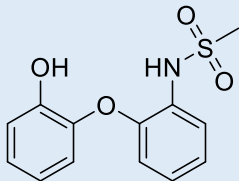
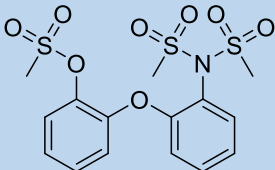
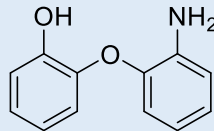
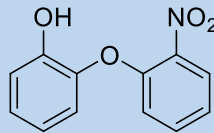
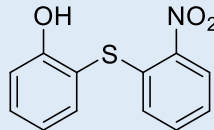


**Scheme 4.1.** Synthesis of KAAI040. The nitro group on KAAI029 was reduced using standard reduction methods. KAAI035 was globally sulfonylated with excess sulfonyl chloride. The sulfonyls on KAAI038 were selectively cleaved under basic conditions, followed by acid workup, to yield the resulting sulfonamide, KAAI040.



**Scheme 4.2.** Synthesis of KAAI048. The nitro group on KAAI028 was reduced using Zinc and acetic acid. KAAI044 was globally sulfonylated with excess sulfonyl chloride. The sulfonyls on KAAI046 were selectively cleaved under basic conditions, followed by acid workup, to yield the resulting sulfonamide, KAAI048.

**Table 4.3.** Percent inhibition of MtFBA at 25 $\mu$ M in fluorescence assay. Inhibitors were tested for inhibition at a concentration of at 25 $\mu$ M. The assay provides a fluorescent signal corresponding with the percent inhibition of the compound on the enzyme.

ID	Structure	Formula	% inhibition at 25 $\mu$ M in fluorescence assay
KAAI048		C <sub>13</sub> H <sub>13</sub> NO <sub>3</sub> S <sub>2</sub>	5.50 $\pm$ 0.30
KAAI046		C <sub>15</sub> H <sub>17</sub> NO <sub>7</sub> S <sub>4</sub>	6.90 $\pm$ 0.10
KAAI044		C <sub>12</sub> H <sub>11</sub> NOS	7.80 $\pm$ 0.60
KAAI040		C <sub>13</sub> H <sub>13</sub> NO <sub>4</sub> S	5.50 $\pm$ 0.50
KAAI038		C <sub>15</sub> H <sub>17</sub> NO <sub>8</sub> S <sub>3</sub>	5.30 $\pm$ 1.70
KAAI035		C <sub>12</sub> H <sub>11</sub> NO <sub>2</sub>	7.00 $\pm$ 0.20
KAAI029		C <sub>12</sub> H <sub>9</sub> NO <sub>4</sub>	9.30 $\pm$ 5.20
KAAI028		C <sub>12</sub> H <sub>9</sub> NO <sub>3</sub> S	6.00 $\pm$ 0.04

Unfortunately, none of the compounds provided significant MtFBA inhibition (Table 4.3). Interestingly the highest percent inhibition came from KAAI029 ( $9.30 \pm 5.20$ ), an early precursor in the synthesis. But with the significant standard deviation, the highest inhibition may be KAAI044 ( $7.80 \pm 0.60$ ), a precursor aryl-amine. Neither of these compounds were the final sulfonamide containing compounds, but rather, intermediates in the respective syntheses.

## **CONCLUSION**

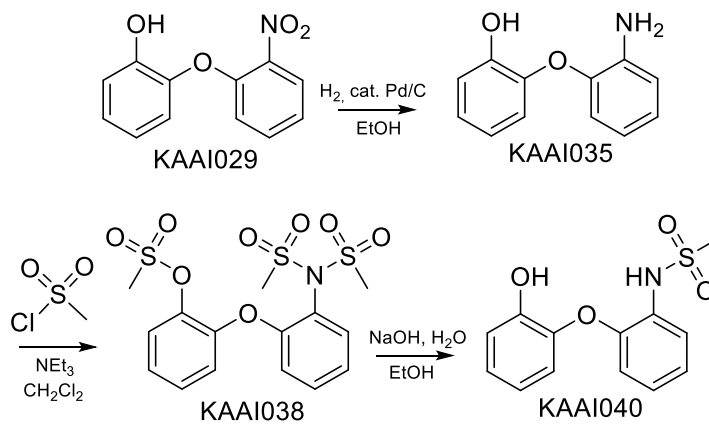
The sulfonamide containing compounds, KAAI048 and KAAI040, do not provide sufficient inhibition of MtFBA to be considered as potential therapeutic agents. Thus, biaryl-sulfonamides may not be good scaffolds for MtFBA inhibition. Alternatively, other unique scaffolds will be explored. For example, HCA has been identified as an MtFBA inhibitor<sup>10</sup> thus future compounds may be modeled after HCA.

## EXPERIMENTAL

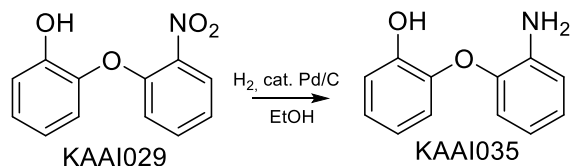
### Preparation of potential MtFBA inhibitors

Proton NMR spectra were recorded at 60 MHz.

### Preparation of KAAI040 (Scheme 4.1)



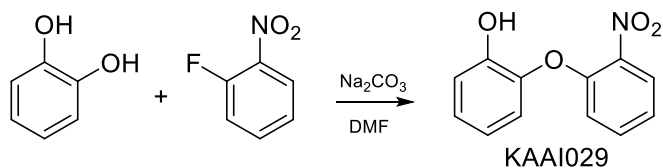
**Scheme 4.1.** Synthesis of KAAI040 via nitro reduction, sulfonylation, and hydrolysis.



**Scheme 4.3.** Nitro reduction of KAAI029 to yield an amine KAAI035.

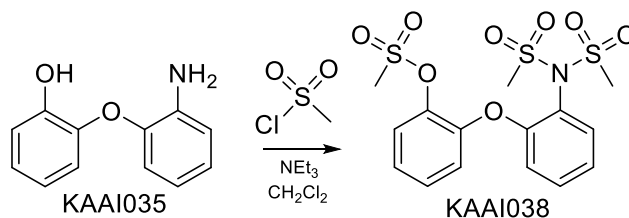
**KAAI035 from KAAI029<sup>Δ</sup> (Scheme 4.3).** Nitrogen gas was blown over a solution of KAAI029 (2g, 8.65mmol) in 43mL of ethanol. Water (~3mL) was added to obtain a 0.2M solution. 2% weight Pd/C (0.9204g, 0.433mmol) was added to the flask followed by excess hydrogen gas. The solution stirred for 24 hours. KAAI035: 48.5% yield. <sup>1</sup>H NMR (60 MHz, CDCl<sub>3</sub>): δ 7.074-6.708 (8H, mult.), 4.8-4.3 (1H, broad sing.).

<sup>Δ</sup>KAAI029 was obtained from Dr. Kateri Ahrendt (Regis University). Synthesized via nucleophilic aromatic substitution. (Scheme 4.4) <sup>1</sup>H NMR (60 MHz, CDCl<sub>3</sub>): δ 7.6-6.8 (4 H, mult.)



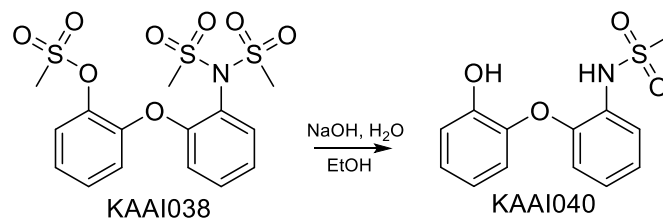
**Scheme 4.4.** Synthesis of KAAI029 via nucleophilic aromatic substitution.





**Scheme 4.5.** Global sulfonylation of KAAI035 to yield KAAI038

**KAAI035 from KAAI038 (Scheme 4.5).** To a suspension of KAAI035 (0.5g, 2.48mmol) in  $\text{CH}_2\text{Cl}_2$  (~10mL, ~0.25M),  $\text{NEt}_3$  (~1.12mL, 8.2mmol) was added. The resulting solution was cooled to  $0^\circ\text{C}$ . Mesylchloride (0.6mL, 7.75mmol) was added dropwise. Within 1-2 min a white precipitate started to form, presumably  $\text{NEt}_3\cdot\text{HCl}$  salt. TLC taken at 5 minutes (30% Ethyl Acetate/Hexanes) was streaky but showed no ninhydrin stain suggesting no remaining Ar- $\text{NH}_2$ . The mixture was stirred for 2 days. The resulting suspension was filtered, extracted using dichloromethane and water, and concentrated using rotary evaporation. KAAI038: 19.5 %yield  $^1\text{H}$  NMR (60 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.5-7.0 (8H, mult.), 3.479 (6H, sing.), 3.163 (3H, sing.)

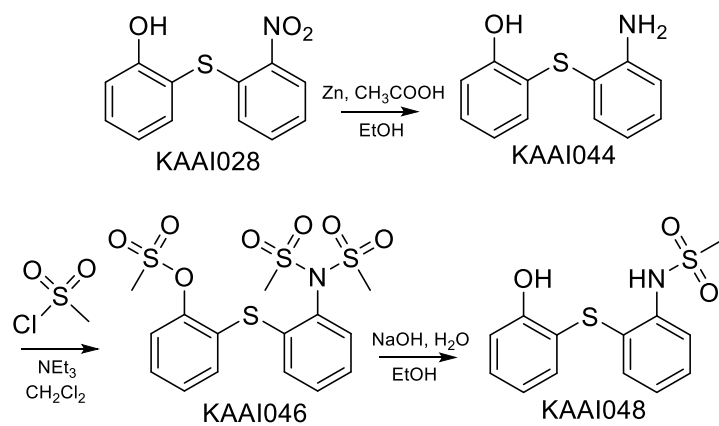


**Scheme 4.6.** Selective sulfonyl cleavage of KAAI038 with base followed by acidic workup to yield KAAI040.

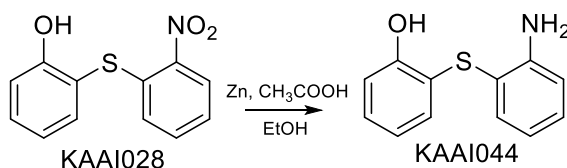
**KAAI040 from KAAI038 (Scheme 4.6).** To a solution of KAAI038 (0.5g, 1.15mmol) in methanol (~4mL, ~0.3M), sodium hydroxide (~4mL, ~0.3M) was added and stirred at 60°C for 24 hours. Hydrochloric acid (~4mL, 0.3M) was added to neutralize the base and protonate the product. The remaining methanol was boiled off and the resulting sticky residue was extracted in 40 ml CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was washed with brine and dried with sodium sulfate, concentrated, and washed with diethyl ether three times. The residue remained as an oil and ~2mL CH<sub>2</sub>Cl<sub>2</sub> was added and let evaporate to obtain the product. KAAI040: 86% yield (0.275g product, 0.985mmol). H<sup>1</sup> NMR (60 MHz, CDCl<sub>3</sub>): δ 7.256-6.812 (7H, mult.), 3.793 (1H, sing.), 3.364 (1H, sing.), 3.067 (3H, sing.), 2.941

(1H, sing.). Not completely pure. No starting material inferred by the upfield shift of aromatic hydrogens. Possible other sulfonylations.

### Preparation of KAAI048 (Scheme 4.2)



**Scheme 4.2.** Synthesis of KAAI040 via nitro reduction, sulfonylation and hydrolysis.

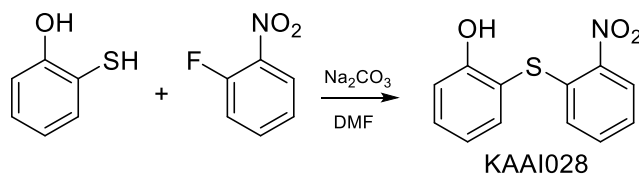


**Scheme 4.7.** Nitro reduction of KAAI028 to yield an amine, KAAI044

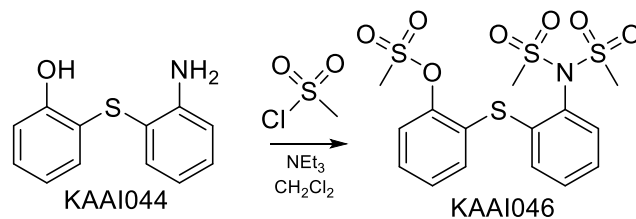
**KAAI044 from KAAI028<sup>Δ</sup> (Scheme 4.7).** To a slurry of Zinc dust\* (1.99g, 31mmol) in 30 mL of 95% alcohol (85% ethanol) and acetic acid (7mL) at 0°C, KAAI028 (1.5g, 6.1mmol) was added. The slurry was heated and stirred at 60°C for 8 hours. The resulting product was filtered with celite and washed with 95% alcohol. The resulting solution was concentrated and an orange solid was obtained. The solid was then extracted using CH<sub>2</sub>Cl<sub>2</sub> and dried using sodium sulfate. The solution was then concentrated and an orange solid was collected. KAAI044. 90%yield: (1.2g) TLC (20% EtOAc/Hexanes) after 4 hours of stirring was visible with ninhydrin indicating Ar-NH<sub>2</sub> formation. H<sup>1</sup> NMR (60 MHz, CDCl<sub>3</sub>): δ 7.520-6.678 (8H, mult.)

\*Reduction with Pd/C did not proceed presumably due to the sulfur poisoning catalyst. Modified procedure from (19).

<sup>Δ</sup> KAAI028 was obtained from Dr. Kateri Ahrendt. Synthesized via nucleophilic aromatic substitution (Scheme 4.8). H<sup>1</sup> NMR (60 MHz, CDCl<sub>3</sub>): δ 8.216 (1H, mult.), 7.518-6.869 (8H, mult.), 6.291 (1H, sing.)

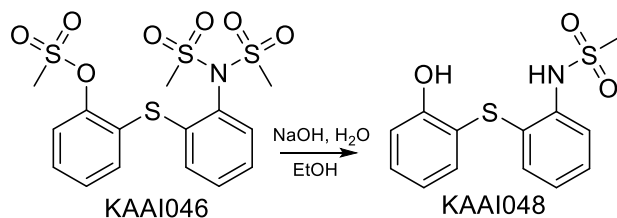


**Scheme 4.8.** Synthesis of KAAI028 via nucleophilic aromatic substitution.



**Scheme 4.9.** Global sulfonylation of KAAI044 to yield KAAI046.

**KAAI046 from KAAI044 (Scheme 4.9).** To a solution of KAAI044 (0.5g, 2.30mmol) in  $\text{CH}_2\text{Cl}_2$  (12mL, ~0.25M)  $\text{NEt}_3$  (1.4mL, 10.1mmol) was added. The solution was cooled to  $0^\circ\text{C}$ . Mesylchloride (0.75mL, 9.66mmol) was added to the solution. After about two minutes a precipitate started to form, presumably  $\text{NEt}_3\cdot\text{HCl}$  and other salts. TLC (20% EtOAc/Hexanes) after 5 minutes of stirring at room temperature was streaky and showed no ninhydrin spots assuming no Ar- $\text{NH}_2$  remaining. The resulting solution was concentrated and a brown solid was obtained. KAAI046. 96%yield:  $^1\text{H}$  NMR (60 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.570-7.185 (8H, mult.), 3.53 (5H, sing.), 3.181 (3H, sing.)



**Scheme 4.10.** Selective sulfonyl cleavage of KAAI046 with base followed by acidic workup to yield KAAI048.

**KAAI048 from KAAI046 (Scheme 4.10).** To a suspension of KAAI046 (0.5g, 1.1mmol) in 4mL 95% alcohol (85% ethanol) 4mL aqueous NaOH (0.193g, 4.3mmol) was added. The resulting suspension was heated and stirred at 60°C for 12 hours. 3M HCl was added to neutralize the base and protonate the product. The product was extracted with CH<sub>2</sub>Cl<sub>2</sub> and water. The solution was concentrated and a brown oil was recovered. Trituration provided ~10mg of solid. (NMR of solid was too dilute to determine if desired product). KAAI048. 75% yield. H<sup>1</sup> NMR(oil) (60 MHz, CDCl<sub>3</sub>): δ 8.14-6.17 (8H, mult.), 3.91 (1H, trip.), 3.31 (2H, sing.).

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## **CONCLUSION**

The previous chapters have primarily been informative regarding a problem affecting antibiotic research and development. However, this chapter presents a perspective on the best course of action for the presented problem besides, or with further emphasis on, the avenues presented in Chapter 3. The antibiotic R&D issue discussed is not one with a clear antagonist. Although big pharma has decreased their investment in antibiotics, these companies are not necessarily to blame. They have simply pursued a business plan that will provide a sound financial return for the company and the investor. Currently, investment in blockbuster drugs is appealing financially. With this in mind, what is the most beneficial path to pursue in greater depth to address the matter in question?

There are many different avenues that can be pursued (Chapter 3), but there are areas that should receive special attention. First, the increase in antibiotic resistance should persuade some big pharma companies to return to antibiotics as there is now a greater need for these drugs. Second, there needs to be increased publicity for bacterial infections such as TB. Third, the clinical trial process needs to be revised to alleviate extraneous costs. Fourth, the next generation of medicinal chemists needs to be inspired to simply do science. However, proposed solutions will never amount to much unless there is significant collaboration.

## HOPE? RESISTANCE CREATES MARKET

The increasing state of antibiotic resistance may actually encourage companies to return to antibiotic R&D. In the mid 1900's infectious diseases were prevalent causing a strong interest from the pharmaceutical industry to develop antibiotics. Antibiotics were greatly needed as some of these diseases rose to epidemic levels. As a result there was a strong market demand for antibiotics.

Currently, increased drug resistant infections, related morbidity, and a lean pipeline all push for the development of new antibiotic agents<sup>1</sup>. With increased antibiotic resistance there is an increase in the market for research and development of novel antibiotics. This is promising as R&D in this field is swinging back to the Gram-negative strains<sup>2</sup> as Gram-negative bacterial infections, such as TB, are exhibiting greater antibiotic resistance. Increased resistance has created a promising market opportunity for the pharmaceutical industry as the small market leads to high prices and the prospect of investment return for these companies<sup>2</sup>. Shales<sup>3</sup> coined the phrase "markets create resistance and resistance creates markets". The lack of funding for antibiotics has accelerated the problem of antibiotic resistance therefore increasing the market for research in this field, increasing the potential for the pendulum to swing back towards antibiotic R&D.

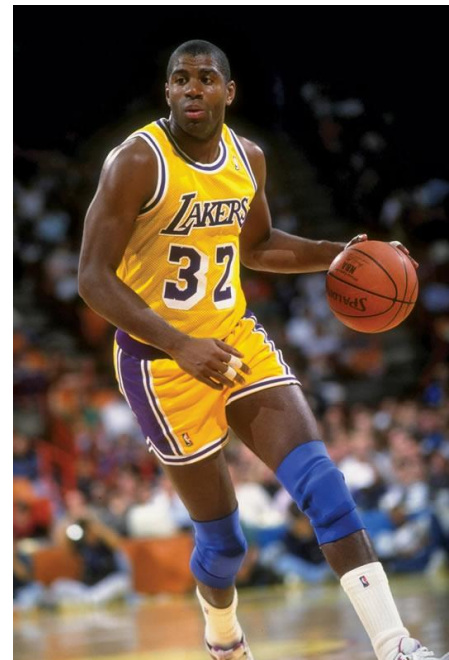
Antibiotic resistance will encourage drug makers to invest in antibiotics. But, that is not the ideal incentive for companies to return to antibiotics. Big pharma is the discovery engine that can make real progress in the development of new antibiotics. They have the financial capital and resources to focus on antibiotic research and development. Should these companies allocate resources towards needed areas? Does big pharma need to see an epidemic to be willing to allocate resources towards antibiotics? An epidemic may encourage big pharma to refocus their attention towards antibiotics, however this perverse incentive should not be why new antibiotics are created. However, this may be the most effective incentive for big pharma.

## HIV/AIDS PUBLICITY

How can needed collaboration be achieved? A small solution is increased publicity for bacterial diseases or specifically TB. It is unlikely to find an individual that does not think that the TB epidemic is a problem once the facts are presented before them. But, he/she may not originally understand the scale of the disease and the main problems involved in treating it because the disease is not publicized to a significant extent.

Despite increasing efforts and progress in the fight against tuberculosis, the disease still remains one of the top worldwide killers<sup>4</sup>. As previously mentioned TB is a disease associated with poverty. Comparably, human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), was once considered a disease primarily associated with poverty, and it remains as one of the top ten global killers<sup>5</sup>. TB and HIV/AIDS are similar in global prevalence; however, TB is not as highly publicized in the United States as the HIV/AIDS epidemic.

Because of the increased occurrence of HIV/AIDS in the United States among celebrities, the epidemic was thrust into the spotlight, causing it to receive significant support across the nation. Earvin “Magic” Johnson (Figure 5.1), the former Los Angeles Lakers basketball player, is arguably the most famous person diagnosed with HIV/AIDS. The disease caused him to retire from the Lakers, halting his career as an athlete<sup>6</sup>. Magic received a request to go public about his condition from AIDS activist Elizabeth Glaser, who contracted the disease from a blood transfusion while giving birth<sup>6</sup>. Since then, he has been a major advocate for the disease. He created the Magic Johnson Foundation, which gives teens college scholarships, hosts



**Figure 5.1.** Earvin “Magic” Johnson. Former Los Angeles Laker, current HIV sufferer and major advocate. Founder of the Magic Johnson Foundation. Adapted from: <http://www.howard.edu/newsroom/releases/2013/20130128EntrepreneurBasketballGreatMagicJohnsonontoDiscussBusinessandHIVStigmaatHowardUniversityHospital.html>

job fairs, and health fairs. One of Magic's main goals is to educate today's young women so that they do not make the same mistakes he did.

"We're able to touch the community in so many different ways. And it's very important to me that we do that and that people of color and the minorities that live in urban America know that they can come to Magic Johnson Foundation and Magic Johnson Enterprises for anything"<sup>6</sup>.

Magic's celebrity status helped in publicizing HIV/AIDS in the United States. Contrarily, the most famous cases of TB include the late Nelson Mandela, Tina Turner, and Ringo Starr<sup>7</sup>. Magic Johnson was a basketball player in the height of his career in the NBA. He had the money and the fame to become a large advocate for the disease. Nelson Mandela, arguably the most famous person affected with TB, was the president of South Africa. He is an advocate for social justice and primarily works with third world issues.



**Figure 5.2** South Africa's former president contracted TB in 1988 while imprisoned in Pollsmoor Prison in Cape Town. Adapted from: (7).

Additionally, HIV/AIDS has received high publicity because of the nature of the disease. It should be noted that HIV is a viral disease, therefore it is not cured as easily as a bacterial infection. Magic Johnson still stresses that after twenty years of fighting it, he is still not cured of HIV. But, if a person contracts TB in the United States, they can be cured in approximately six months

with the current drug regimen. Just four months after Mandela contracted TB he was cured<sup>8</sup>. Although this a long treatment for a bacterial disease, it is still significantly shorter than the twenty year fight Magic has been facing. Magic advocates for HIV/AIDS to prevent the future development of the disease in the United States. However, there is not

the same need for this type of advocacy for TB in the U.S. as the spread of disease can be avoided and it is still primarily a third world disease. But rather, the need for advocacy in the United States is to focus on treatments and ways to prevent patient compliance issues so that this can be addressed in developing countries where these issues are of greater concern.

## **CLINICAL TRIALS**

Clinical trials are an obvious necessity for the development of a new antibiotic. The focus on ameliorating the antibiotic R&D funding burden should be on alleviating extraneous costs<sup>9</sup> specifically in regards to the cost of clinical trials.

From 1994 to 2003 funding for biomedical research more than doubled from \$37.1 billion to \$94.3 billion<sup>10</sup>. In that time funding for clinical trials in the pharmaceutical industry increased from 37 to 64% of total biomedical research costs, but FDA approval of new molecular entities decreased by approximately 33% during that time<sup>10</sup>. This lack of productivity indicates that clinical trial practices are not as efficient as they need to be in drug development, therefore they need to be reassessed to decrease costs and increase efficiency.

In a 2008 study, a panel of experts was hired to design clinical trial simulations to decrease costs. The team created two types of clinical trials; one that followed standard budgetary protocol and another that aimed to decrease costs by using a streamlined industry model. The main panel recommendations for the streamlined model includes “(1) increasing the ability of sites to be top performers; (2) using computer systems to improve site management and monitoring, and (3) streamlining and enhancing clinical trial operations”<sup>10</sup>. Recommendation 1 includes ideas such as using site facilities that best meet protocol requirements. Recommendation 2 includes ideas such as remote monitoring including conference calls and in house monitoring to decrease travel costs. Recommendation 3 includes ideas such as evaluation of cost-effectiveness of current practices with further research<sup>10</sup>.

With the expert recommendations and a streamlined industry model, the cost of clinical trials was decreased by 68%. These recommendations could save the developing

companies hundreds of millions of dollars. Based on these results, reevaluation of clinical trials to decrease costs is a promising avenue that should be pursued further.

### **INSPIRING SCIENCE**

Chemists working at pharmaceutical companies are not intentionally ignoring antibiotics. They are working to do chemistry, because it is their passion. If the next generation of medicinal chemists can be inspired, then they will perform research to do science, and ideally not get caught up in the business plan. This is where academic research can play a role. Students that chose to pursue graduate studies are those that have decided they want to pursue research, at least for the time they are in graduate school. Antibiotic research should be pursued to a greater extent in the academic setting because of the students' desire to learn. Because graduate school is primarily about learning and time is not as much of a factor, there is more freedom on which research path to pursue and a decreased pressure for production as compared to industry. If the students are inspired to do research at the graduate level, then they will hopefully take this inspiration with them as they enter the work force as medicinal chemists.

### **THE CHALLENGE OF SCIENCE**

The majority of the previous writing has been devoted to addressing the issue of funding for antibiotic R&D. Even if these proposed solutions, and other previously existing ones, could solve the funding issue, there are still additional challenges to overcome. The main additional challenge is science. Even if scientists receive adequate funding, that does not necessarily mean that they will produce any significant results. This is because in reality science is hard. There are two main categories of rate limiting steps to the antibacterial discovery process. The first is identifying proper molecular targets, specifically ones that are not prone to resistance. The second is the limitation of chemical diversity, especially ones that are effective against Gram-negative organisms<sup>11</sup>. Within those categories include topics such as the types of antibiotic resistance, the challenge of discovering new classes of antibiotics, and many other complicated scientific issues.

The point is that the lack of novel antibiotics is inherently more complex than just funding. There is no cure all for the issue. But, steps can be taken in more specific areas such as publicity, assessing clinical trials, and inspiring science. In all of these

collaboration needs to be an integral part. Only with collaboration can small steps be made in the perpetual fight against bacteria.

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