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„TACKLING ANTIBIOTIC RESISTANCE IN *M. TUBERCULOSIS:* DESIGN AND SYNTHESIS OF NOVEL ANTITUBERCULAR SMALL MOLECULES“

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Georg Bánó

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TACKLING ANTIBIOTIC RESISTANCE IN *M. TUBERCULOSIS*: DESIGN AND SYNTHESIS OF NOVEL ANTITUBERCULAR SMALL MOLECULES

GEORG BÁNÓ

KING'S COLLEGE LONDON

SUPERVISOR: DR. DANIELE CASTAGNOLO

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Abstract

As the cause of 1.7 million deaths in 2016[1], *M. Tuberculosis* (MTB) represents a serious global threat to those infected. Growing numbers of multidrug-resistant (MDR) specimen increase the urgency of improving existing treatment, as well as

[1] (WHO, 2017)
[3] (Bhakta, et al., 2016)

developing new mechanisms of inhibition. This study covers an approach of tackling MDR- *M. Tuberculosis* by studying antibacterial effects of newly synthesized chlorophenylthiazoles and indole derivatives.
Although not fully described, previously synthesized structures, that are model for structures described in the following[3,4], show good inhibitory activity in MTB and mycobacterial efflux pump inhibitory (EPI) activity, allowing a high expectancy of finding lead structures active in infections with MDR-Tuberculosis (MDR-TB)[5]. Thus this work aims to build a narrow chemical library as an asset for further research.

1. Introduction

1.1.World wide threat

Tuberculosis was the first disease to be declared a global health emergency by the WORLD HEALTH ORGANIZATION (WHO) in 1993 (WHO, 1994), aiming to counter an incline of reported cases after a period, in which the disease was believed to be under control due to new chemotherapy in 1940s and implementation of an adequate therapy scheme, directly-observed therapy short course (DOTS) in the 1980s (Ramakant, 2009).
In spite of these achievements, tuberculosis, listed amongst the ten deadliest diseases, requires treatment for millions of people annually. About 10,5 million people fell ill only in 2015 (WHO, 2017).
Including 480 000 patients with multidrug-resistant infections of *M. Tuberculosis*.
Although the bacteria is a global threat, 60% of its incidences are concentrated in only 6 countries worldwide with India leading the count, followed by Indonesia, China, Nigeria, Pakistan and South Africa (WHO, 2016).
This shows the disease is an even bigger concern in developing regions as high population density and low hygienic standards promote its spread.
Figure 1. shows the epidemic is heavily plaguing African and South Asian regions with a share of 86% of all cases (WHO, 2009).
The connection between poverty and high incidences of TB is well recognized also strengthening the thesis of malnutrition weakening the immune system and therefore leading to a promotion of contagion (Zaman, 2010).
Despite clinical awareness and precaution, MDR-TB and RR-TB (rifampicin resistant tuberculosis) infections are globally – although on a level below 1% - still on an increasing trend from 2015 to 2016 (WHO, 2017). Due to the statistic bias this moderate trend only accounts for a global average including all countries. Whereas viewed only within WHO's 30 countries with high MDR-TB burden, already nine of these were challenged with an increase of MDR-TB cases by 30% from 2015 to 2016 (WHO, 2017).

[3] (Bhakta, et al., 2016)
[4] (Scalacci, et al., 2017)
[5] (Bhakta, et al., 2016)

1.2.Tuberculosis

Diagnosed as a bacterial infection by *Mycobacterium Tuberculosis,* mainly affecting the lungs, tuberculosis is responsible for 6% of all annual deaths worldwide, thus dealt as the deadliest infection by a single agent pathogen (WHO, 2016) (Marwick, 1992), (Wilson, Braunwald, Isselbacher, & al., 1991; Mc Neil & Brennan, 1991).

Contagious by its airborne germs, TB can be spread by coughing, spitting, talking or sneezing from one individual to another (CDC; National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention Division of Tuberculosis Elimination, 2011). Untreated a manifested infection lasts about 3 years until death or self-cure (Tiemersma, 2011). Typical symptoms of pulmonary TB are nausea, fever, a general feeling of weakness, night sweats, unintended weight loss, pain in the chest, chronic coughing and even coughing up blood (Zaman, 2010).

If not treated, the final cause of death of pulmonary TB is a septic shock due to vast pulmonary infection (Chou-Han, 2014).

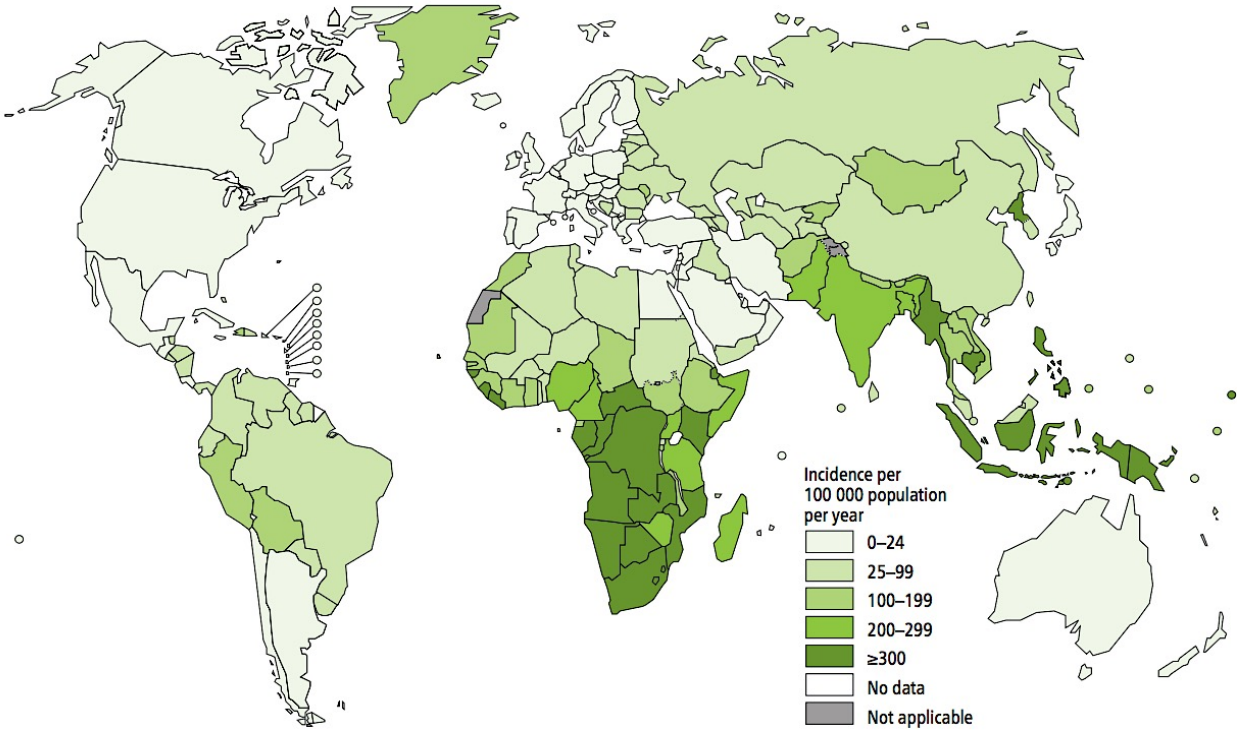


Figure 1. Estimated TB incidence rates in 2016**, (WHO, 2017)**

The malady has a characteristic progress divided into two phases, the latent phase (inactive or closed TB) and the open phase (active or open TB) (Chou-Han, 2014). During the latent phase the immune response of infected patients is strong enough to prevent the proliferation and outspread of further bacteria, however the immune system is not able to fully eradicate the mycobacteria. Therefore fibrocytes, lymphocytes, macrophages and *M. Tuberculosis* germs live in an inflammatory

equilibrium of proliferation and necrosis, forming a granuloma, which is sited on the inner, lower tissue of the lungs (Schluger, 2017).

The latent form is non-contagious to other individuals. This state can last for years or even decades without showing any signs of illness. Once the immune system is weakened by age, human immunodeficiency virus (HIV), malnutrition or similar negative influences, the vivid *M. Tuberculosis* germs burst out of the outer layer of fibrocytes, proliferate, affect broader parts of the lung and induce the active or open form of tuberculosis, which then is highly contagious.

Once *M. Tuberculosis* has penetrated the lungs it is ingested by alveolar macrophages into a phagosome. Regarding this standard procedure of the human immune system, the threat of *M. Tuberculosis* lies within its ability to prevent the fusion of lysosomes with phagosomes within the macrophages by producing a prohibiting protein (McKinney, 2013). Therefore hydrolytic enzymes cannot reach the phagocyted bacteria and lysis can't be induced. Using this mechanism of survival *M. Tuberculosis* inhibits its own degradation, by not allowing the lysosomatic proteases to reach the bacteria within the phagosome (Lemke & Williams, FOEY's Principles of Medicinal Chemistry 7th Edition, 2013).

In a case of closed TB the cells within the granuloma eventually will die, leaving marks of caesous necrosis and a calcinated area as an evidence of a cured infection. This mark is called tuberculome. It is an area in the pulmonary that is visible in X-Rays of patients that suffered from TB at some point during their life, sometimes without even noticing (Kumar V, 2005).

The importance of the immune system in preventing an outburst of tubercular pathogens, is shown especially in patients suffering from immune deficiency, such as Acquired Immune Deficiency Syndrome (AIDS) patients, in which symptoms of active TB show within weeks after incubation (WHO, 2016). In 2015, 55% of TB patients globally had a documented HIV test result (WHO, 2016).

Furthermore as an estimate of 1/3 of the world's population is considered to carry a latent TB infection (LTBI) it is also possible that affected patients with a high auto immune response can live with a latent infection, not suffering from any symptoms of illness or restrictions during their lifetime. The risk of activating the latent form into open TB during a lifetime is estimated with 5% to 10% (WHO, 2015).

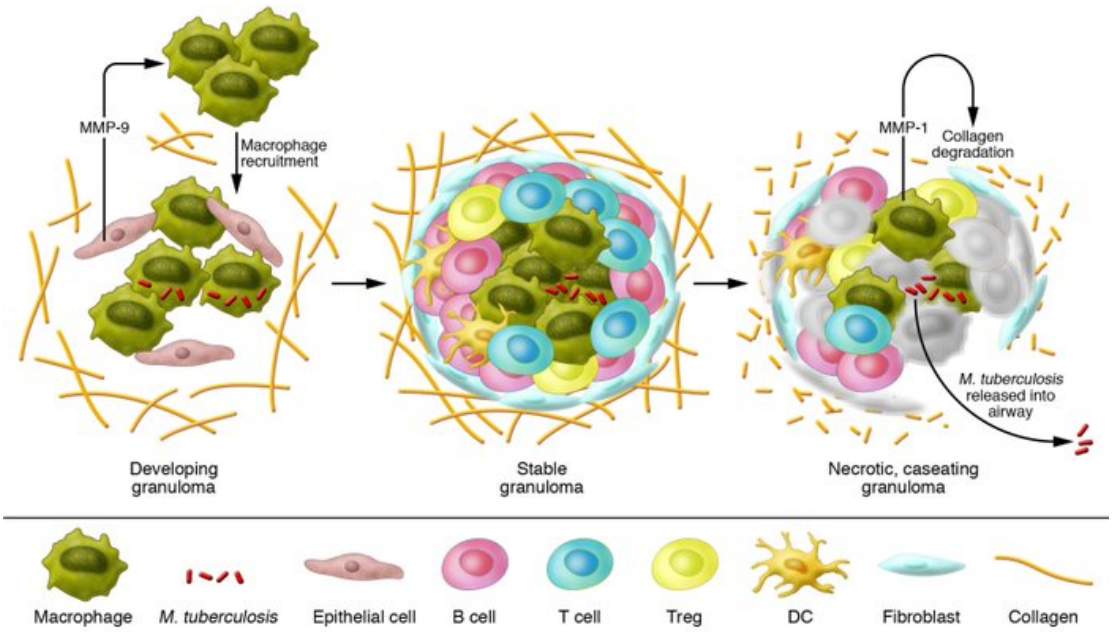


Figure 2. . Development of a granuloma **(Salgame, 2011)**

M. Tuberculosis can also affect extra pulmonary regions such as the brain, intestines, kidneys, or the spine, causing extra pulmonary tuberculosis (EPTB), by facilitating the transport via the vascular system such as, sterile pyuria affecting the kidneys, meningitis affecting the brain, Addison's disease affecting the adrenal glands or hepatitis affecting the liver (Ji Yeon Lee, 2015). Extra pulmonary manifestations of tuberculosis are less common with a share of only 20% of all TB cases reported (Ekaterina Kulchavenya, 2014).

1.3. Mycobacterium Tuberculosis

M. Tuberculosis is a rod-shaped, non-spore-forming, aerobic bacilli, counting to the family of acid-fast, gram-negative bacteria (Porth & Kunert, 2002). Its typical measures are 0.5 µm by 3 µm, allowing microscopic determination. The cell wall is lipophilic, due to a high amount of the fatty acid, mycolic acid attached to arabinogalactan, a polysaccharide bound to the underlying peptidoglycan layer (Lee, Li, Chatterjee, & Lee, 2005). The unique biochemical composition results in its many challenging characteristics such as medical and immunological resistances. It is persistent to alcohol, acids and alkali, leaving the bacilli in a bright red color when previously treated with a Ziehl Neelsen stain (Rastogi, 1991). Accordingly when MTB is colored with a basic dye, it will resist decolorization (Rastogi, 1991) (Minnikin, 1991).

Due to its waxy cell wall it can persist weak disinfectants and survive on dry surfaces for several months (Brennan, 2003). The untypical exterior mantle provides *M. Tuberculosis* with sufficient mechanisms of survival in the human body and therefore also seems to be an interesting target for future research.

Aiming especially on the membrane stated bacterial efflux pump also seems to be a likely promising approach (Brennan, 2003).

In average the germs of *M. Tuberculosis* double every 24 hours to 38 hours and therefore is a respectively slow process compared with other bacteria (James, Williams, & Marsh, 2000).
This also makes biological testing, such as structure activity relation (SAR) testing challenging, as generating results can take up to several weeks, due to slow growth.

2. Diagnosis & treatment

2.1. Diagnostics

In times of consistently increasing numbers of TB incidences it is very important to have accurate and fast methods of screening susceptible and high risk patients and identify those infected.
First of all radiographs of the chest can provide typical findings of infiltrates with cavitation in the upper area of the lungs indicating active tuberculosis (Thrupp, Bradley, Smith, Simor, & Gantz, 2004). Especially patients with compromised immune system, such as elderly or autoimmune deficient patients may not show these typical findings as their immune response is too weak.
Therefore the sputum smear is a very traditional way to detect the presence of acid-fast bacilli in patients with untypical characteristics in their radiographs. Three sputum will be collected on three consecutive days, separately smeared on a slide, then stained and dried. In the last step the dried sputum will be tested for its color persistency and is treated with alcohol. In presence of any mycobacteria the bacilli will remain red as it does not destain (Centers for Disease Control and Prevetion, kein Datum).
Although this method gives information about bacterial infection, it does not distinguish whether it is *M. Tuberculosis* or any other mycobacteria.
To have proof for the diagnosis of tuberculosis the clear identification of *M. Tuberculosis* in a culture, taken from the sputum of a patient is required. This approach can take three to six weeks due to the slow growth of the bacteria (Nancy A. Knechel, 2009).
Another alternative is a high-performance liquid chromatography isolating the mycolic acid compounds giving confirmation of infections in about two weeks (Centers for Disease Control and Prevetion, kein Datum).
Newer techniques include the amplification of DNA and RNA by polymerase chain reaction providing definite outcomes within hours. Although this is a technically very advanced method its low availability, low sensitivity and high costs are big disadvantages for common use (Mazurek GH, 2005).

As mentioned the latent or closed tuberculosis does not show any symptoms of illness nor does the sputum of infected patients contain any tubercle pathogens.
Therefore the methods listed above do not qualify for identifying any form of latent infection.

The tuberculin test (also Mantoux tuberculin test) is the most common screening method for latent or active *M. Tuberculosis.*
For this process purified protein derivative (PPD) - 0.1mL of intermediate-strength – containing five tuberculin units as an antigen is injected into the skin of susceptible patients (Nancy A. Knechel, 2009).
A positive result will show as a cell-mediated immune reaction after 48 to 72 hours, as hardening and reddening of the site of tuberculin penetration occurs. Typically the inner forearm is used for testing.
The disadvantage of the tuberculin test is, that it only tells whether any mycobacteria has ever been present in a patient or not. It does not give any information about whether the infection is still present and if it is latent or active. Considering false negative outcomes, immunocompromised patients may only show no or a heavily delayed immune response after two to ten weeks leading to misinterpretation and failing to isolate and treat infected. Also false positive results can occur in patients that suffered from any mycobacterial infection other than tuberculosis (Nancy A. Knechel, 2009).
In 2005 the QuantiFERON-TB Gold test, an alternative for testing for latent tuberculosis infections was introduced.
It includes taking a blood sample of whole blood and incubating it with a tubercle antigen. After sufficient time to produce a possible cell mediated immune response the amount of leukocyte-released interferon-γ is measured utilizing an enzyme-linked immunosorbent assay (Nancy A. Knechel, 2009). This method is providing reliable results about latent and active tuberculosis within less than 24 hours (Mazurek GH, 2005).

2.2. Treatment

Once the suspicion of tuberculosis has been confirmed, isolation of infected patients is required. It is recommended to place them into negative-pressured rooms and minimize the number of visitors. To prevent the disease from spreading, visitors are required to wear particulate respiratory masks whenever entering the isolated area. Respectively patients leaving the room must also be advised to wear a mask covering their mouth and nose (Toth, Fackelmann, Pigott, & Tolomeo, 2004).
Besides the urgency of medical treatment, observations show a higher risk for patients with very low body-mass-index to have higher incidences of relapse, than those with high body-mass-index. Improvement of nutrition and body weight by about 5% during the first two months of infection are considered sufficient to significantly lower the risk of relapse (Khan, Sterling, & Reves, 2006).

2.3. Treatment of drug susceptible tuberculosis

Although the mentioned above is beneficial in contribution to full recovery and constrain the epidemic, intensive and well monitored medicinal treatment is crucial for the eradication of the disease and the survival of patients.

Following the robust nature and ability of *M. Tuberculosis* to inhibit phagocytosis by macrophages, even effective drug therapy lasts for six to nine months. During this long period the compliance of patients can decrease, and irregular drug intake can promote drug resistance. There are also several other scenarios in which risk of building resistance is higher. These include persons previously treated with antitubercular medicine, patients and hospital staff working in institutions exposed to multi-resistant strains and people living in high-risk countries. The WHO therefore recommends medical regimens implementing directly observed therapy (DOT) (WHO, 2017). Rather than leaving the responsibility of correct drug intake in the own hands of patients, DOTs distribute the delivery and drug intake to nurses (clinic based DOT) or to trained personnel at work, home, or elsewhere in the community (community based DOT).

In the 1994 guidelines of the US Centers for Disease Control and Prevention and the American Thoracic Society five antitubercular drugs, isoniazid, rifampicin, pyrazinamide and a combination of streptomycin and / or ethambutol are listed as first line treatment. This recommendation is still valid and characteristics of mentioned drugs will be discussed more precise in the following (Bass, et al., 1994). Rifampicin, showing to be the drug most responsible for a fast response of the disease and to be an important asset for shorter treatment regimens, is combined with isoniazid. Together they are forming the most crucial mix of drugs. Nevertheless observations show that an additional introductory phase of ethambutol or streptomycin or both, for two months can predict a positive outcome for 95% of all treated patients. In order to reduce the time of drug intake, the implementation of pyrazinamide into the mix is favorable, as it can reduce the treatment period to six months only, still retaining positive outcomes for 95% of in such way treated patients (Hong Kong Chest Service/British Medical Research Council, 1993). Patients with persistent resistance (most commonly against isoniazid) can reduce the chance of developing an additional resistance to rifampicin by adding ethambutol to the treatment regimen (Bass, et al., 1994).

2.4. Treatment of tuberculosis and HIV coinfections

Whereas patients with ordinary clinical picture have good chances of an uncomplicated eradication of germs, patients with HIV or multidrug resistant specimen need customized treatment regimen to control their progression of disease.

Antiretroviral medication is inhibiting the growth of CD4 positive lymphocytes compromising their immune system furthermore (WHO, 2017). In most cases this can lead to worsening of untypical findings in pectoral radiographs such as worsening of infiltrates, swelling on lymph nodes or pericardial effusions. Precautions can be taken by delaying antiretroviral treatment until patients have undergone several months of antituberculotic treatment. Antiretroviral therapy (ART) should be implemented as soon as possible within the first eight weeks of antituberculotic treatment, regardless of their CD4 cell count. It is recommended to start ART already within the first two weeks of treatment in patients with very low immune response

(e.g. CD4+ cell count below 50 cells/mm^3) (WHO, 2017). HIV positive patients also have an increased risk of relapse (Pulido, et al., 1997).
The treatment of multidrug-resistant specimen is not less problematic. It is most commonly the result of poorly organized treatment practices (Mahmoudi & Iseman, 1993).

2.5. Treatment of multidrug resistant tuberculosis

Patients with infections that prove to be resistant against at least isoniazid and rifampicin should be treated with a minimum of five drugs considered as effective against the specimen. In the case of multidrug resistant (MDR) or rifampicin resistant (RR) tuberculosis patients "short-term" treatment regimens (lasting nine to twelve months), instead of "long-term" treatment regimens (lasting longer than 18 months) are recommended (WHO, 2016). The short-term treatment is divided into two separate parts, firstly the intensive phase enduring four to six months, depending on the success of sputum conversion and secondly the continuation phase of five months. The fist, intensive phase includes gatifloxacin (or moxifloxacin), high-dose isoniazid, pyrazinamide, prothionamide, kanamycin, clofazimine, and ethambutol (WHO, 2017). Whereas the second, continuation phase includes only gatifloxacin (or moxifloxacin), clofazimine, pyrazinamide and ethambutol. The benefits of shorter regimens had been widely overseen as a result of lacking evidence, but recent publications show that statistically significant improvements could be achieved by shortening the length of treatment (WHO, 2016). If success was compared to failure of treatment, death or relapse 90% of shorter regimens succeeded, versus 78% succeeding in longer regimens (WHO, 2016). The 2016 update of the WHOs tuberculosis report 2016 replaces the qualifications of the older, longer regimens (Chan & Iseman, 2002) as the conventional standard by these new revelations.
Treatment of latent and active Tuberculosis can last for six to nine month, or even longer. The long duration of the available treatment decreases compliance and patients may stop treatment before full eradication of all infectious germs. Thus bad compliance and the spread of resistant bacteria from patients with LTBI are reasons for the increase of MDR- and XDR-TB (extensively drug resistant TB) (Velayati, 2009).
Although the share of MDR- and XDR-TB only lies within approx. 4% of all new TB infections and 20% of previously treated TB, its death rate lies at about 50%, still causing a serious threat to those infected (WHO ANNEX 3, 2017).
Regarding resistance against the best first line drug, rifampicin, 16% of all new cases and 60% of all previously treated cases of MDR-TB show notified resistance when treated with rifampicin (WHO ANNEX 3, 2017).
As stated above MDR-*M. Tuberculosis* specimen can also be found in patients never treated with antitubercular medicines, making the threat even less controllable (Migliori, 2007).
The overall list of drugs, some strains of *M. Tuberculosis* may be resistant to, is growing lager every year (WHO, 2010).

2.6. Medication

2.6.1. First line anti-TB drugs

For treatment of drug-sensitive tuberculosis, i.e. no drug-resistance to be observed, only first line agents should be used. They outstand for their high effectiveness, low adverse effects and high clinical experience. Current treatment regimens use four drugs. Although streptomycin is only injectable, an implementation into therapy should be desired. Rifabutina and rifapentinea are also considered first line oral antituberculosis drugs (WHO, 2016).

2.6.1.a. Isoniazid

It is the hydrazide of isonicotinic acid, a derivative of pyridinecarboxylic acids. It is only bactericidal to fast replicating mycobacteria, whereas it is only bacteriostatic to slow growing germs (WHO, 2017). It is usually formulated for oral intake and penetrates all fluids and liquids. One of its targets is the catalase-peroxidase (KatG), which is responsible for oxidizing electron donors, such as NADP(H), which is most notably important for its survival in macrophages as it is withstanding the phagocyte oxidative burst (Johnsson & WA Froland, 1997) (Sherman, et al., 1996).

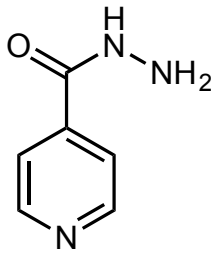
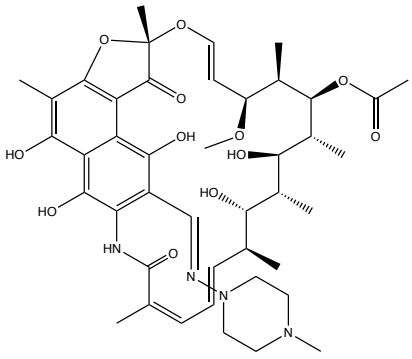


Figure 3. Chem. structure of Isoniazid (Jensen, 1954).

2.6.1.b. Rifampicin

Rifampicin is the semisynthetic derivate of rifamycin, derived of streptomyces mediterranei. It is a makrolactam counted as an ansamycin antibiotic. Physiologically it interacts with DNA dependent RNA-Polymerase forming an adduct. By this mode of action it inhibits the initiation of RNA-synthesis and thus is bactericidal in a wide range of mycobacteria (Goodman, Gilman, Hardman, & Lee, 1996). It is applied orally, best 30 minutes before meals, as its resorption is decreased with simultaneous food intake. After resorption it is distributed into body fluids and cellular tissues (WHO, 2017). To counter increasing resistance against rifampicin it is advised to combine it with other effective antimycobacterial agents (WHO, 1991).

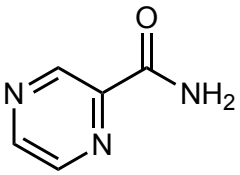


rifampicin

Figure 3. Chem. structure of Rifampicin (Ibiapino, Pitaluga, Trindaded, & Ferreira, 2014)

2.6.1.c. Pyrazinamide

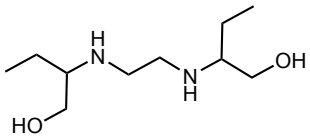
Pyrazinamide is a synthetic analogue derived of nicotinamide (WHO, 1991). Only effective in non-replicating persisters, it plays an important role in macrophages, sterilizing persistent bacilli other antitubercular therapeutics fail to kill (Ying, Wanliang, Wenhong, & Mitchison, 2013). Applied orally it is well absorbed by the gastrointestinal tract. It is very important during the first two months of treatment where inflammatory periods persist. The implementation of pyrazinamide shortens the length of therapy and lowers the risk of relapse (WHO, 1991).



pyrazinamide

2.6.1.d. Ethambutol

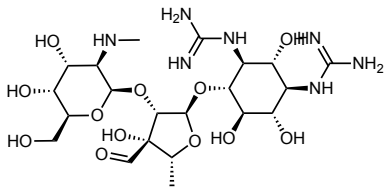
Ethambutol is a synthetic alcohol of 1,2-ethanediamid (WHO, 1991). Ethambutol is bacteriostatic, antituberculotic and antimicrobic. By inhibiting the biosynthesis of arabinogalactan, a major component in *M. Tuberculosis* it interferes with a correct composition of the cell wall in the mycobacteria. This is done by blocking the arabinosyl transferases (Smith & Reynard, 1992). Such as the other first line drugs it also is taken orally. It should be especially included into therapy when primary resistance to other antitubercular agents is suspected.



ethambutol

2.6.1.e. Streptomycin

Streptomycin is an aminoglycoside antibiotic binding the ribosomal 30S subunit and inhibiting the crucial steps of initiation and elongation of protein synthesis (Heding, 1967). It is active in gram-positive and gram-negative bacteria and therefore a broad-spectrum antibiotic (Schantz & Ng, 2004). It is produced by the actinomycete Streptomyces griseus. Streptomycin is not absorbed by the gastrointestinal tract and only available by parenteral or intramuscularly injection (Kucers, Crowe, & Grayson, 1997).



streptomycin

2.6.2. Second line anti-TB drugs

Second line antituberculotic drugs are mainly used to treat infections with *M. Tuberculosis*, that are not sensitive for first line treatment. This could be the cause of previous treatment or exposure with isoniazid, rifampicin, pyrazinamide, ethambutol or streptomycin for longer than one month and result in drug resistance. Second line

treatment will also be used in complicated coinfections such as HIV and tuberculosis or therapy of relapse (WHO, 2016).
Besides first- and second line drugs the WHO recommends the further distinction of anti-TB drugs into four groups (update of 2016 guidelines for treatment of drug-resistant tuberculosis).

2.6.2.a. Group A – Flouroquinones

The fluoroquinones levofloxacin, moxifloxacin and gatifloxacin a group of gyrase inhibitors, a bacterial topoisomerase enzyme, are the most valuable assets in eradication persistent drug-resistant tuberculosis strains. By inhibiting the unwinding of DNA, respectively the reattachment of separated DNA strains they inhibit the replication of bacteria (Hooper, 2001). Although moxifloxacin and other fluoroquinones may prolong the QT-interval, they have a good overall safety profile. Oxafloxacin and ciprofloxacin are excluded for indication as evidence cannot prove their effectiveness (WHO, 2016).

2.6.3.b. Group B – second line injectable

It is advised that one of the following aminoglycoside agents amikacin, capreomycin, kanamycin and in some cases streptomycin is always included in the treatment regimen. They are most likely to lead to treatment success, especially in long term TB treatment.
Although streptomycin is not considered to be a second line agent its use in MDR-TB is recommended if other injectable agents fail and there is no known resistance to streptomycin (WHO, 2016). As for most aminoglycoside antibiotics they can cause ototoxicity leading to deafness and nephrotoxicity leading to renal impairment (Prayle, Watson, Fortnum, & Smyth, 2010).

2.6.4.c. Group C - Other core second-line agents

As evidence shows success of treatment is higher when at least four different medicines are included in the core regimen during the intensive care. The main purpose of ethionamide / prothionamide, cycloserine / terizidone, linezolid and clofazimine is to supply sufficient drug agents to achieve the minimum count of five drugs (WHO, 2016).

2.6.5.d. Group D - Add-on agents

These medicines are not considered to be part of the core treatment regimen. Similar to group C, the main purpose of group D drug agents is to widen the range of available anti-tuberculotic medicines. They have a relatively low effect in tuberculosis, however they are beneficial in infections in which first- and second line medication is not active. They are divided into three subgroups D1 (pyrazinamide, ethambutol, high-dose isoniazid), D2 (bedaquiline, delamanid) and D3 (p-aminosalicylic acid, imipenem–cilastatind, meropenemd, amoxicillin-clavulanate) (WHO, 2016).

3. Experimental part

3.1.Chemistry – Synthesis

The herein synthesized structures are separated into two generations of derivatives, the first to be *p*-Cl-phenylsubstituted cycloalkylaminothiazoles and the second to be cycloalkylaminoindoles.

The activated *p*-Cl-phenylthiazolocarbaldehyde was obtained already activated at sigma-Aldrich, therefore only step two of the following general synthesis was performed (**scheme 1.**).The second series was synthesized as the following.

In a two step reaction indole **3a**, 5-chloroindole **3b**, 6-chloroindole **3c** and 5-methylindole **3d** were first carboxylated in position 3 via Vilsmeier-Haack reaction.

In the second step the aldehyde moiety of **3a, 3b, 3c** and **3d** was used to perform a reductive amination and add N-substituted cycloalkylamines with six to eight fully saturated ring members (**scheme 2.**).

In detail, the indoles **3a-d** were first reacted with a cooled solution of phosphorous oxychloride ($POCl_3$) and N,N-dimethylformamide (DMF). DMF was supplying the formamide entity to yield the indolecarbaldehydes **4a-d**. In the last step the respective amines (i.e. cyclohexylamine, cycloheptylamine, cyclooctylamine, exo-2-aminonorbonane or cyclohexanemethanamine) were treated with acetic acid (AcOH) and sodiumtriacetoxyborohydride (STAB, NaB $(AcO)_3H$) as a reductive agent to obtain the final products **2a** and **b**, **5a-j** with satisfactory, but lower-than-average yields (36% to 86%).

The compounds **2a** and **b** were inspired by N-((1-(4-Chlorophenyl)-2,5- dimethyl-1H-pyrrol-3-yl)methyl)-cyclohexanamine **7** (Bhakta, et al., 2016), a compound synthesized in 2016, that was found to have high activity in *M. Tuberculosis*. The minimal inhibitory concentration (MIC) was as low as 0.2 µg/ml in *M. Tuberculosis* subtype H37Rv and 0.5 µg/ml in the multidrug resistant subtype MDR1 (Bhakta, et al., 2016). To investigate the influence of heteroatoms on the biological activity the pyrrole ring of **7** was replaced by a thiazole moiety introducing sulfur into the structure. This led to the first series of derivatives aiming to determine the influence of heteroatoms on the overall biological activity.

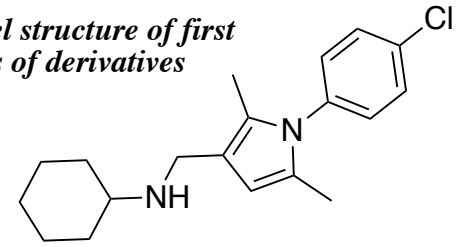
In both series of derivatives the chlorine substituent was particularly included to observe its importance to structure related activity in Cl-substituted compounds **5e-h** and compare it to those compounds in which chlorine was absent **5a-d** or was replaced by a methyl group **5i** and **j**.

Another aspect of investigation was the influence of bulky substituents on the activity of synthesized compounds. Compared with 1-(2-(piperidin-2-yl)ethyl)-1H-indole **6,** a compound inspired by the structure of thiaridozine (Scalacci, et al., 2017), compounds **5a-j** have been further modified by replacing the piperidine with cycloalkylamines. Hence the introduction of voluminous carbocycles with as many ring members as possible was intended. At a certain magnitude of about nine ring

members the steric hindrance of carbocycles stopped the addition of cyclic moieties larger than eight atoms. Undertaken synthesis with such reagents therefore failed.

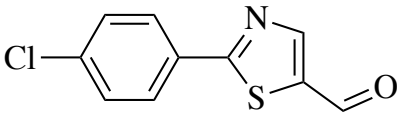
3.1.1. FIRST SERIES OF DERIVATIVES – CHLOROPENYLTHIAZOLES

Model structure of first series of derivatives



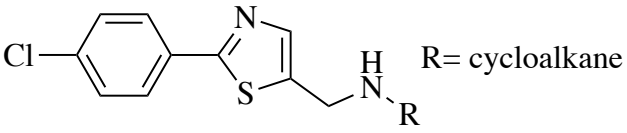
N-((1-(4-Chlorphenyl)-2,5-dimethyl-1H-pyrrol-3-yl)methyl)-cyclohexanamine (Bhakta, et al., 2016).

basic structure

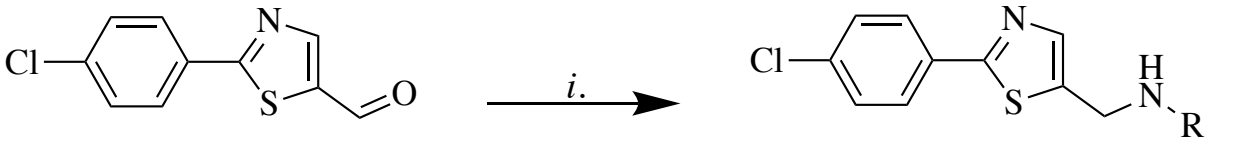


2-(4-chlorophenyl)thiazole-5-carbaldehyde

addition of cycloalkylamines



first series of derivatives



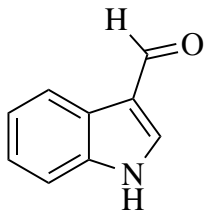
1

2a, R= cyclohexyl
2b, R= cycloheptyl

Scheme 1. Synthesis of analogue **2**. Reagents and conditions: i. THF, AcOH, NaB(AcO)3H, 1M NaOH, 18 h, cyclohexylamine for **2a,** or cycloheptylamine for **2b**.

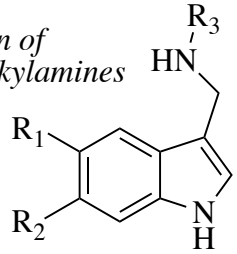
3.1.2. SECOND SERIES OF DERIVATIVES - INDOLES

basic structure



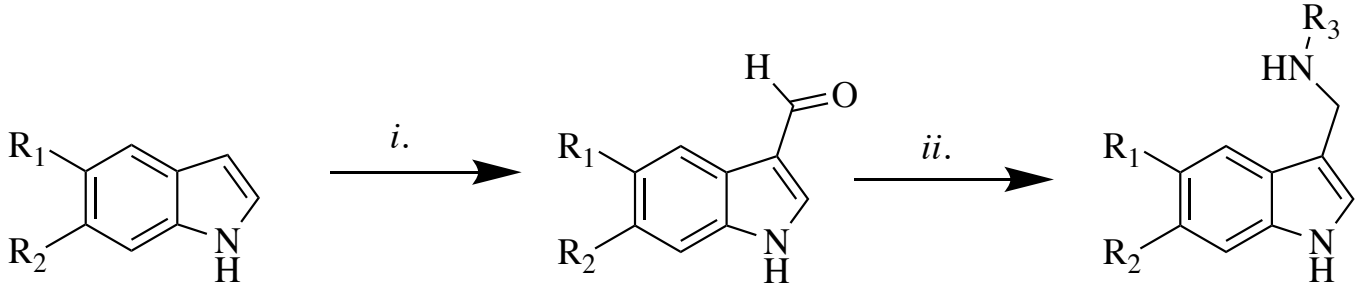
1H-indole-3-carbaldehyde

addition of cycloalkylamines



R_1= H, Cl, Me
R_2= H, Cl
R_3= cycloheptylamine, cyclooctylamine, exo-2-aminonorbornane, cyclohexanemethylamine

second series of derivatives



3a, R_1= H, R_2= H
3b, R_1= Cl, R_2= H
3c, R_1= H, R_2= Cl
3d, R_1= CH_3, R_2= H

4a, R_1= H, R_2= H
4b, R_1= Cl, R_2= H
4c, R_1= H, R_2= Cl
4d, R_1= CH_3, R_2= H

5a-j

Scheme 2. Synthesis of analogues **5a-j**. Reagents and conditions *i*. DMF, $POCl_3$, 0°C, 2h; *ii*. THF, AcOH, $NaB(AcO)_3H$, 1M NaOH, 18 h, cycloheptylamine for **5a**, **f**, **h** and **j**, or cyclooctylamine for **5b,** or exo-2-aminonorbornane for **5c**, or cyclohexylmethanamie for **5d**, or cyclohexylamine for **5e, g** and **i.**

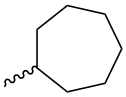
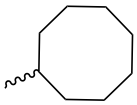
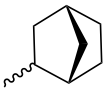
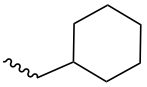
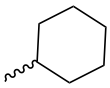
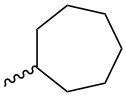
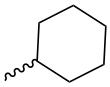
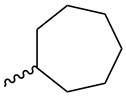
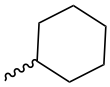
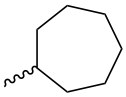
Cmpd.	R_1	R_2	R_3
5a	H	H	
5b	H	H	
5c	H	H	
5d	H	H	
5e	Cl	H	
5f	Cl	H	
5g	H	Cl	
5h	H	Cl	
5i	CH_3	H	
5j	CH_3	H	

Table 1. Substituents of compounds **5a-j.**

3.2. Previous chemical considerations

The design of these novel structures was also inspired by the model of the antimycobacterial structure of **8,** BM212 (Deidda, Lampis, Fioravanti, Biava, & Porret, 1998) (Biava, et al., 2006) and the likewise antitubercular structure of **9,** SQ109 (Protopopova, et al., 2005). In spite of the mycobacterial growth inhibiting activity of these two model compounds, they were lacking satisfying pharmacological profiles (Jia, et al., 2005). A combination of functional and structural moieties of **8,** BM212 and **9,** SQ109 unveiled potent pyrrole hybrid derivatives (Bhakta, et al., 2016). Mimicking the structural arrangement of in this way found compound, N-((1-(4-Chlorphenyl)-2,5-dimethyl-1H-pyrrol-3-yl)methyl)-cyclohexanamine, **7** with a MIC of 0.2 µg/ml against *M. Tuberculosis* H37Rv (Bhakta, et al., 2016) led to the hypothesis of finding activity in similar structures. Therefore one

chlorophenylthiazole and substituted or unsubstituted indoles have been synthesis for the objective work.

3.3. Chemical background, The Vilsmeier-Haack reaction

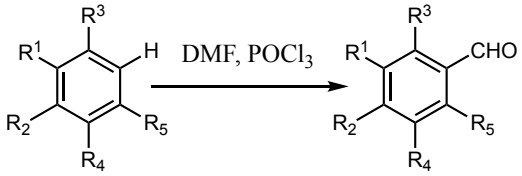


Figure 3. Vilsmeier-Haack reaction scheme, R=Any **(Vilsmeier & Haack, 1927)**.

The Vilsmeier-Haack procedure creates aromatic or aliphatic aldehydes of electron rich aromatic and aliphatic substrates serving as reactants. The Vilsmeier-Haack reagent is a weak electrophile and is formed insitu, treating N,N-dimethylformamide (DMF) with an acid halide ion, usually phosphorous oxychloride ($POCl_3$) forming chloromethyleneiminium salts. For attachment on the target the desired aromatic substrate is added to the reactive mixture of DMF and $POCl_3$ bearing the iminium ion. Now due to its positive charge of the annealed quartery nitrogen, this unstable functional group on the aromatic ring represents a good target for hydrolysis. After addition of H_2O the hydrolysis of the present iminium salt affords aldehyde derivatives coupled to the respective desired aromatic cycle.

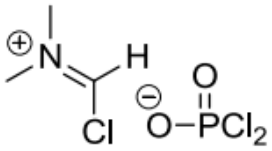


Figure 4. Vilsmeier-Haack reagent **(Li, 2014)**.

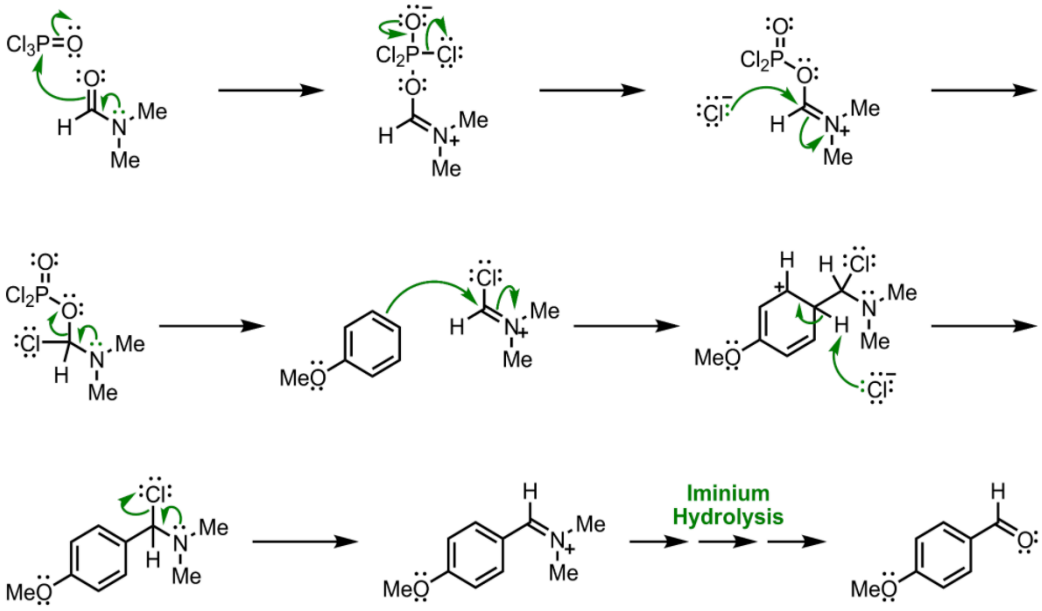


Figure 5. Reaction mechanism of Vilsmeier-Haack formylation **(synarchive.com, 2017)**

4.DISCUSSION

Investigating on a new lead structure active in infections with MDR-TB, the goal of the objective thesis has been the synthesis of a narrow library of one chlorophenylthiazole derivative and N-substituted Indole derivatives, following the structural principles of **8,** BM212 and its inhibiting activity of the bacterial trehalose monomycolate exporter protein MmpL3 (Rv0206c) (La Rosa, et al., 2012) (Scalacci, Pellojab, Radic, & Castagnolo, 2016).

The chemical structure of thioridazine, a neuroleptic drug which indication was broadend to also be used in the treatment of tuberculosis, has been recent subject of research to enhance its activity and cytotoxicity profile (Scalacci, et al., 2017). The base body of synthesised compounds **5a-j** was inspired by compound t-Butyl 2-(2-(1H-indol-1-yl)ethyl)piperidine-1-carboxylate, **6** (Scalacci, et al., 2017). **8,** BM212 and the Adamantan-diamine compound **9,** SQ109 (Prototopova, et al., 2005) were also showing inhibition on the membrane stated efflux pump (Bhakta, et al., 2016). Thus antimycobacterial activity is expected in combination with available antitubercular drugs.

The main effort lied in synthesizing structures very close related to those mentioned above, to gain further knowledge about structural requirements to increase biological activity of drug agents against MDR-TB strains.

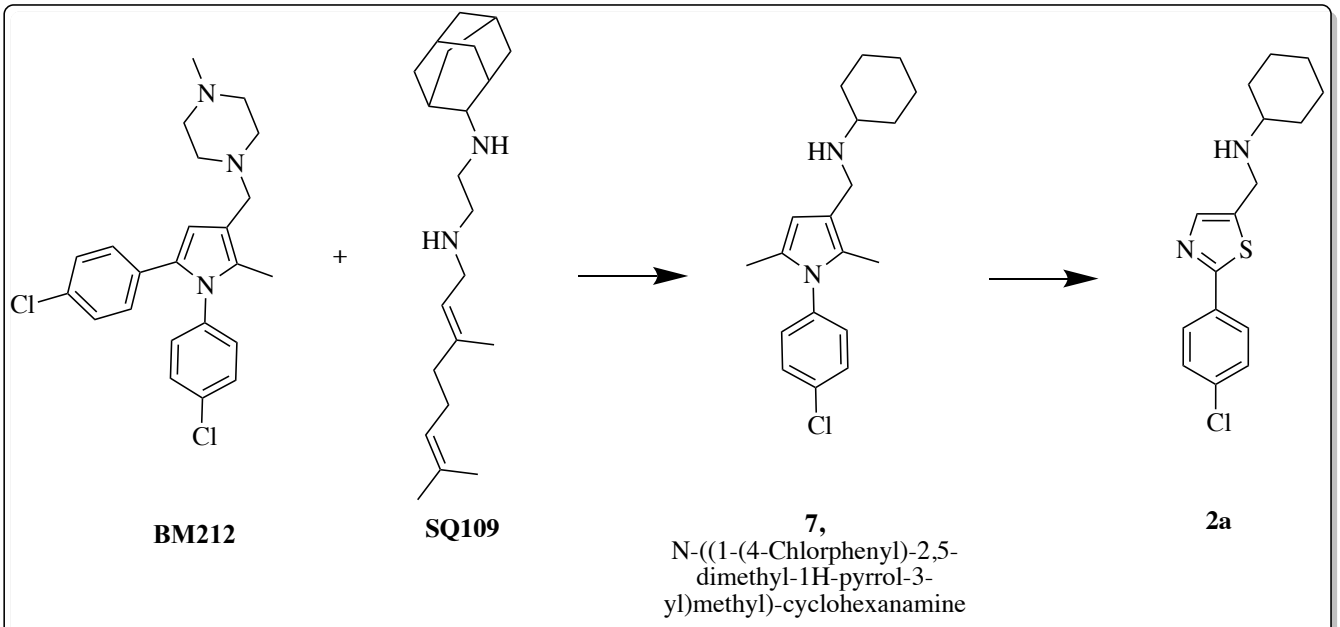
Reductive amination produced a range of different yields with the lowest being 12% and the highest 76%.

The exceptional low yield of 12% must be an outlier due to poor interpretation of in process monitoring and therefore early termination of the still ongoing reaction. As already stated the behavior and the detection of the respectively synthesized compounds is tempting for such errors, as the indole based compounds have very high polarity and are only poorly eluated. Considering the bulky structure of more than seven to eight carbon atoms of some of the added cycloalkaneamines, the low reaction yields of 12% **(5f)**, 36% **(5d)**, can also be interpreted as a result of steric hindrance, therefore the reactants not reaching the reactive site of the aldehyde moiety. For this reason addition of even bigger cycles was not attempted. Therefore the approach of synthesis is not to be considered as inadequate for the objective purpose. A repetition of reactions with very low yield was not attempted as the absolute amount of every synthesized substrate was still vastly sufficient for planned biological SAR testing.

The approach of working at room temperature or with assistance of an oil bath at approx. 40°C seems to deliver results with an average completion rate, bearing the average yield of approx. 80% for reductive aminations in mind. Nevertheless it leaves reaction times well over 2 days. This fact makes this way of synthesis a very slow

process. In order to obtain a high through put of synthesized structures, this approach demands setting up several reactions parallel to each other, which may result in a higher chance of unintended errors or less exactness in monitoring reactions leading to secondary products or decreasing yields.
Thus, compared with procedures using microwave assistance (for example for benzofuranes (Vincetti, et al., 2016)) to form substituted 2-aminothiazoles (Scalacci, Pellojab, Radic, & Castagnolo, 2016) which are able to accomplish similar quantitative outputs in only several minutes, the use of microwave assisted procedure schemes is highly recommended if available.

As a previous work (Bhakta, et al., 2016) shows, deleting or modifying substituents on **8,** BM212 or **9,** SQ109 could improve their anrtitubercular activity as well as their cytotoxicity profile. It was observed that compounds that replaced their N-methylpiperazine functionality by cycloalkylamines as well as lost their *p*-Cl-phenyl substituent at C5 by replacement with a methyl group showed significantly low MICs of 0.2 µg/ml or 0.5 µg/ml (Bhakta, et al., 2016).
In detail the *p*-Cl-phenyl group, the distal nitrogen of the piperazin moiety and further distanced bulky N-substituents like adamantyl on the piperazin showed to have negative influence on the antimycobacterial activity. Nevertheless a linear amino spacer in a very similar overall size like cyclohexylamine was mandatory to obtain activity of synthesized derivatives. This was confirmed by decreasing activity when introducing a larger substituent like a phenylethylamino spacer instead.
N-((1-(4-Chlorphenyl)-2,5-dimethyl-1H-pyrrol-3-yl)methyl)-cyclohexanamine, **7** therefore showed the highest activity against MTB H37Rv with a MIC of 0.2 µg/ml (Bhakta, et al., 2016). Due to the structural homology of **7** and **2a** the hypothesis of **2a** being very active as well strengthened.
On the other hand, high antitubercular activity was not correlating with a high efflux pump inhibitory (EPI) effect. Therefore structurally similar compounds to **7**, bearing bulky substituents like alkyl groups on the piperazine ring showed high EPI effects. This feature also promotes the advantages of derivatives of **7**, which are model for **2a**, in comparison with BM212, **8** and SQ109, **9,** which only show inhibition of the transporter pump MmpL3 (Bhakta, et al., 2016). Following this chemical attributions, although lacking the piperazine moiety, **2a** gives hope for also having EPI activity.
The efflux pump is a protein stated in the mycobacterial membrane transporting antimycobacterial agents into the extracellular lumen, thus promoting drug resistance. Hence combination of compounds with EPI could be an important future asset to standard therapy to tackle drug resistance in MTB.



Scheme 3. Previous structures leading to **2a.**

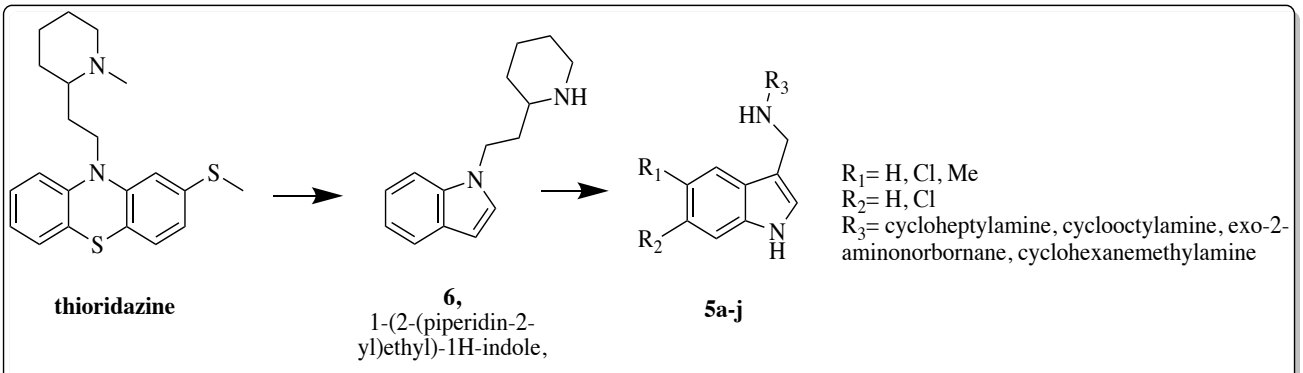
Compared with the second series of compounds, **5a-j**, which were inspired by 1-(2-(piperidin-2-yl)ethyl)-1H-indole, **6,** they are structurally more different to **7** and **2a** , but still share important core structure features like the 5 member aromatic amino heterocycle (pyrrole in **2a**, and indole in **5a-j**) and the cycloalkylamine substituent.

Comparison of their antimycobacterial effect will further elucidate the importance and functionality of substituents (other than the cycloalkyamines on C3) on the pyrrole ring, if conceptually indole is considered as a 2,3 benzopyrrole.

A previous work (Scalacci, et al., 2017), observed **6,** an indole bearing a 2-ethylpiperidine in position N-1, to show MICs of 2.9 µg/ml against the pathogenic TB strain H37Rv and 1 µg/ml against the drug susceptible CF73 clinical isolate (Scalacci, et al., 2017). As described in scheme 4, following this revelations the second series of derivatives of this work only differ by the position of substitution on the indole, namely substitution on N for **6** and position 3 for **5a-j** as well as the position of the nitrogen of the piperidine to be stated in the alkyl chain (now connecting position 3 of indole and the cycloalkane).

In a cytotoxicity assay **6** showed to have a 15-fold decrease in cytotoxicity tested against MRC-5 cells (Scalacci, et al., 2017).

This characteristics and the described common features of **6, 7** and **5a-j** support the hypothesis that the first and second series derivatives are ought to show good antitubercular activity or efflux pump inhibition with a good cytotoxicity profile.



Scheme 4. Previous structures leading to **5a-j.**

5. CONCLUSION

In total, one novel chlorophenylthiazole derivative **2a** (**scheme 1.)** and ten new indole derivatives **5a-j** were synthesized **(table 1.)**.

The fast reaction time of the formylation of indole respectively of chlorophenyl-5-thiazole of approx. 2h per reaction, makes the use of indolecarbaldehyde respectively chlorophenyl-5-thiazolocarbaldehyde a very convenient starting material for reductive aminations.

As stated above the performed reaction to create the final compound is reliable to synthesize desired structures.

Derived by its model N-((1-(4-Chlorophenyl)-2,5-dimethyl-1H-pyrrol-3-yl)methyl)cyclohexaneamine **7** (Bhakta, et al., 2016) the structural similarity of **2a** is promising some inhibiting activity of MDR *M. Tuberculosis*. In the same way 1-(2-(piperidin-2-yl)ethyl)-1H-indole **6** (Scalacci, et al., 2017) gives good reason to expect the synthesized indole derivatives **5a-j** to show promising inhibitory effects on MDR-TB strain and point the direction for new lead structures of new anti-tubercular agents.

The Limpsinki rule of five was met by all compounds, as they show less than 10 hydrogen bond acceptors, less than 5 hydrogen donors and a molecular weight of less than 500 g/mol. Therefore good pharmacological profiles are to be expected.

SAR testing of newly introduced thiazole and indole derived structures will provide relevant information for future research projects in the pursuit of tackling drug resistant tuberculosis.

6. BIOLOGY - prospective biological experiments

The synthesized compounds have been sent for SAR testing.

To give satisfactory information about the relevance of separate structural elements and their importance to biological activity different tests are planned to be performed in *M. smegmatis*, *M. aurum, M. bovis, M. tuberculosis* and multiresistant clinical isolates of *M. tuberculosis* (MDR1 and MDR2), as well as determination of minimal

inhibitory concentration, cytotoxic analysis in MRC-5 cell lines, cytotoxicity assay and efflux pump inhibition assay.

Due to the slow replication of *M. Tuberculosis* of about 24 h per cycle (James, Williams, & Marsh, 2000), SAR testing of biological activity in the newly synthesized products against MDR *M. Tuberculosis* germs is still in progress.

7. CHEMISTRY-Material and Methods

For recording ^{1}H-NMR and ^{13}C-NMR spectra a Bruker spectrometer at the frequencies 400 MHz and 100 MHz respectively was used. Chemical shifts (∂) are reported in ppm referenced to tetramethylsilane. The reported coupling constants (*J*) are in hertz, rounded to 0.5 Hz. Used abbreviations for splitting patterns are: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br).

Mass-spectra were carried out using positive mode scanning over a range of mass of 50-1500. The used insource parameters were as followed: the flow of drying gas was 9 ml/min; the nebulize pressure was 40 psig; and the drying gas temperature was 350°C.

Flash columns were packed with Fluorochem Davisil 40-63µm, 60Å as stationary phase.

Reactions were carried out in oven dried glassware.

Thin layer chromatography (TLC) was accomplished by using pre-coated plates that where commercially available and visualized with UV light at 254 nm; K_2MnO_4 was used to expose the products.

All reactions have been monitored by thin layer chromatography (TLC) using varying combinations of MeOH, EtOAc and hexane as eluents depending on the polarity of the expected products. Because of their high polarity the desired products had very low Rf-values, leaving them not being sufficiently eluted by conventional eluents to show satisfactory separation. Therefore the best results have been accomplished using a high polarity mixture of MeOH/EtOAc, 1:9 v/v. The desired products were expected at a R*f* of 0 to 0.2 at an eluent grade of EtOAc only or a mixture of MeOH/EtOAc, 1:9 v/v. To avoid disintegration of TLC plates, chromatograms were processed as quick as possible.

The correct substitution of the formed indolealdehydes at position C3 was confirmed with correlation spectroscopy (COSY) and nuclear Overhauser spectroscopy (NOESY).

THF was obtained by distilling it under nitrogen from sodium using benzophenone as an indicator.

Materials obtained from Sigma Aldrich were N, N-Dimethylformamide, $NaB(AcO)_3H$, cyclopentylamine, cyclohexanemethylamine, cyclohexylamine, cycloheptylamine, cyclooctylamine, exo-2-Aminonorbornane, 1-Adamantylamine chlorophenyl-5-thiazolocarbaldehyde, indole, 5-chloroindole, 6-chloroindole and 5-methylindole.

Materials that have been obtained from Fisher Scientific UK were ammonia, dichloromethane, ethanol, ethyl acetate, glacial acetic acid, hexane, methanol and tetrahydrofurane.
Phosphorous oxychloride was obtained from Alafa Aesar.
All structures have been drawn using ChemDraw® Professional (PerkinElmer, Version 17.0.0.206 (131)) unless stated otherwise.

Synthesis of chlorothiazole derivative 2a. *General procedure.* The aldehyde **1** (1 mmol) was dissolved in 5ml of THF in a round bottom flask. Then AcOH (1mmol) and the appropriate amine (1mmol) were added to the stirring solution at room temperature. The mixture was allowed to stir for 20 min before adding NaB(AcO)3H (3mmol). After stirring for approx.18 to 36 h, depending on the desired grade of completion, the reaction was monitored with TLC and was quenched by adding 1M NaOH (12,5ml), if sufficient completion had occurred. For complete termination the solution was allowed to stir for additional 30 min. Next the mixture was diluted with EtOAc (5 ml) and washed twice with EtOAc (5ml) and once with brine (10 ml). After extraction in a separating funnel the organic phases were collected and dried over $MgSO_4$, then filtered through cotton and concentrated under reduced pressure. After concentration the residue was again dissolved in EtOAc (2.5 ml) and purified via flash chromatography (hexane/EtOAc, 1:1 v/v). (Bhakta, et al., 2016).
Each step was monitored by TLC, visualized with UV light at 254 nm. The obtained fractions were monitored with TLC and only those, containing the desired product were collected and again concentrated under reduced pressure.

N-((2-(4-chlorophenyl)thiazol-5-yl)methyl)cyclohexanamine (2a). Yield: 23% ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 7.86 (s, 1H), 7.84 (s, 1H), 7.64 (s, 1H), 7.41 (s, 1H), 7.39 (s, 1H), 4.06 (s, 2H), 2.54 (br. s., 1H), 1.92 (d, J = 12.59 Hz, 4H), 1.74 (d, J = 12.72 Hz, 4H), 1.62 (d, J = 11.33 Hz, 2H) ppm; ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 136.1, 132.0, 129.2, 127.6, 77.4, 77.0, 76.7, 55.6, 41.4, 31.9, 31.7, 29.7, 29.4, 25.5, 24.7, 22.7 ppm; LRMS *m/z* (ES+) *m/z* 307 $[M + H]^+$.

Synthesis of indole aldehyde derivatives (4a-d) *General procedure.*
First $POCl_3$ (6 mmol) was mixed into an ice-cooled round bottom flask containing DMF (5 ml) by dropwise addition. For this reaction, no protective atmosphere containing N_2 was created. After 15 min of assured homogenous distribution of $POCl_3$ in DMF the appropriate indole **3a-d** (1mmol) was added. The reaction was allowed to stir for 2 h at 0°C. The temperature was monitored utilizing a thermostat at all times. TLC was used to track progression of the reaction. After sufficient completion the reaction was quenched by adding a 10% NaOH solution (20 ml), then diluted with EtOAc (10 ml) and purged by washing two times with EtOAc (10 ml) and once with brine (20 ml). The solution was kept stirring until the reaction was terminated.
After extraction in a separating funnel using EtOAc the organic phases were collected and dried over $MgSO_4$, then filtered through cotton and concentrated under reduced pressure (Bhakta, et al., 2016).

After condensation under reduced the residue was again dissolved in EtOAc (2.5 ml) and purified via flash chromatography (hexane/EtOAc, 8:2 v/v) affording the desired products **4a-d**.

1H-indole-3-carbaldehyde (4a). Yield: 86%

Values are confirmed as in literature.

^{13}C (400 MHz, DMSO−d6 + CDCl3) 115.2(C−9);119.1(C−5); 121.7(C−3);121.9(C−7); 127.8(C−6);130.2(C−8); 139.0(C−10);140.1(C−4); 162.0(C−2) (Jain, 2007)

5-chloro-1H-indole-3-carbaldehyde (4b). Yield: 67%

^{1}H NMR (400 MHz, DMSO-d6) δ 12.36 (br. s., 1H), 9.98 (s, 1H), 8.42 (s, 1H), 8.12 (s, 1H), 7.60 (d, J = 8.69 Hz, 1H), 7.34 (d, J = 8.69 Hz, 1H) ppm; ^{13}C NMR (101 MHz, DMSO-d6) δ 185.1, 139.5, 135.4, 126.7, 125.2, 123.5, 119.8, 117.5, 114.1 ppm; LRMS *m/z* (ES+) *m/z* 180 $[M + H]^+$.

6-chloro-1H-indole-3-carbaldehyde (4c) Yield: 60%; values confirm as described in literature.

5-methyl-1H-indole-3-carbaldehyde (4d) Yield: 52%; values confirm as described in literature.

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 10.03 (s., 1H), 9.23 (br. s., 1H), 8.13 (s, 1H), 7.82 (d, J = 1.00 Hz, 1H), 7.35 (d, J = 8.43 Hz, 1H), 7.15 (d, J = 9.82 Hz, 1H), 2.48 (s, 3H) ppm; ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 185.4, 135.9, 135.1, 132.8, 125.9, 124.7, 121.6, 119.2, 111.3, 21.5 ppm; LRMS *m/z* (ES+) *m/z* 160 $[M + H]^+$.

Synthesis of indolecycloalkylamine derivatives (5a-j). *General procedure.* The respective indolealdehyde **4a-d** (1 mmol) was dissolved in 5ml of THF in a round bottom flask. Then AcOH (1mmol) and the appropriate amine (1mmol) were added to the stirring solution at room temperature. The mixture was allowed to stir for 20 min before adding NaB(AcO)3H (3mmol). After stirring for approx.18 to 36 h, depending on the desired grade of completion, the reaction was monitored with TLC and was quenched by adding 1M NaOH (12,5ml), if sufficient completion had occurred. For complete termination the solution was allowed to stir for additional 30 min. Next the mixture was diluted with EtOAc (10 ml) and washed twice with EtOAc (10ml) and once with brine (20 ml). After extraction in a separating funnel the organic phases were collected and dried over $MgSO_4$, then filtered through cotton and concentrated under reduced pressure (Bhakta, et al., 2016).

After condensation under reduced pressure the residue was again dissolved in EtOAc (2.5 ml) and purified via flash chromatography (gradient elution starting from low polarity with only EtOAc, steadily increasing the polarity to EtOAc/MeOH 9:1 v/v). The column was then washed three times with EtOAc (20 ml), then 2 drops of ammonia were added and washed quickly until no product was left in the column. Each step was monitored by TLC visualized with UV light at 254 nm. The obtained fractions were monitored with TLC and only those, containing the desired product were collected and again concentrated under reduced pressure, affording the desired products **5a-j**.

N-((1H-indol-3-yl)methyl)cycloheptaneamine (5a). Yield: 63%

^{1}H NMR (400 MHz, Acetone) d 10.28 (br. s., 1H), 7.68 (d, J = 7.93 Hz, 1H), 7.38 (d, J = 8.06 Hz, 1H), 7.23 (s, 1H), 7.10 (t, J = 7.05 Hz, 1H), 7.02 (t, J = 7.49 Hz, 1H), 3.97 (s, 2H), 2.81 (tt, J = 4.09, 8.18 Hz, 1H), 2.22 (br. s., 1H), 1.91 (m., 2H), 1.69 (m., 2H), 1.49 (m., 8H) ppm; ^{13}C NMR (101 MHz, Acetone) δ 206.4, 137.7, 128.3, 123.7, 122.1, 119.8, 119.4, 115.5, 112.1, 58.9, 43.3, 35.4, 25.0 ppm; LRMS *m/z* (ES+) *m/z* 243 $[M + H]^+$.

N-((1H-indol-3-yl)methyl)cyclooctanamine (5b). Yield: 62%

^{1}H NMR (400 MHz, Acetone) δ 10.22 (br. s., 1H), 7.68 (d, J = 7.93 Hz, 1H), 7.37 (d, J = 8.06 Hz, 1H), 7.23 (s, 1H), 7.05 - 7.12 (m, 1H), 6.97 - 7.04 (m, 1H), 3.96 (s, 2H), 2.77 - 2.86 (m, 1H), 2.34 (br. s., 1H), 1.37 - 1.66 (m, 14H) ppm; ^{13}C NMR (101 MHz, Acetone) δ 206.2, 128.3, 123.7, 122.1, 119.8, 119.4, 112.1, 57.9, 43.2, 32.7, 28.3, 26.4, 24.6 ppm; LRMS *m/z* (ES+) *m/z* 257 $[M + H]^+$.

N-((1H-indol-3-yl)methyl)bicyclo[2.2.1]heptan-2-amine (5c). Yield: 46%

^{1}H NMR (400 MHz, Acetone-d6) δ 10.15 (s, br, 1H), 7.67 (d, J = 7.9 Hz, 1H), 7.37 (dt, J1 = 8.1 Hz, J2 =0.9 Hz, 1H), 7.21 (d, J = 2.2 Hz, 1H), 7.11 – 7.07 (m, 1H), 7.03 – 6.99 (m, 1H), 3.90 (m, 2H), 2.71 (dd, J1 = 7.3 Hz, J2 = 2.3 Hz, 1H), 2.22 (m, 1H), 1.64 (dt, J1 = 9.4 Hz, J2 = 1.8 Hz), 1.56 – 1.43 (m, 3H), 1.22 – 1.17 (m, 1H), 1.11 – 1.04 (m, 3H) ppm;

^{13}C NMR (101 MHz, Acetone) δ 124.7, 123.6, 122.1, 119.8, 119.4, 112.1, 62.3, 55.0, 46.5, 43.7, 41.6, 40.6, 36.5, 35.5, 29.5, 27.5 ppm;

LRMS *m/z* (ES+) *m/z* 241 $[M + H]^+$.

N-((1H-indol-3-yl)methyl)-1-cyclohexylmethanamine (5d). Yield: 36%

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 9.41 (br. s., 1H), 7.87 (d, J = 7.55 Hz, 1H), 7.52 (d, J = 7.93 Hz, 1H), 7.39 - 7.45 (m, 1H), 7.33 - 7.39 (m, 1H), 7.27 (s, 1H), 4.23 (s, 2H), 3.14 (br. s., 1H), 2.83 (d, J = 6.80 Hz, 2H), 1.85 - 2.04 (m, 6H), 1.31 - 1.59 (m, 5H) ppm;

^{13}C NMR (101 MHz, Acetone) δ 128.4, 124.8, 122.2, 122.0, 119.6, 119.2, 112.1, 111.9, 51.0, 45.4, 38.4, 32.2, 27.4, 26.8 ppm;

LRMS *m/z* (ES+) *m/z* 244 $[M + H]^+$.

N-((5-chloro-1H-indol-3-yl)methyl)cyclohexanamine (5e). Yield: 30%

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.62 (br. s., 1H), 6.91 - 7.04 (m, 1H), 6.88 (d, J = 1.00 Hz, 1H), 6.79 (s., 1H), 6.74 (s, 1H), 3.65 (s, 2H), 2.29 (m., 1H), 1.93 (br. s., 1H), 1.23 - 1.80 (m, 1H), 0.80 - 0.89 (m, 3H) ppm;

^{13}C NMR (101 MHz, CHLOROFORM-d) δ 134.7, 128.1, 125.1, 124.2, 122.2, 118.1, 114.2, 112.4, 56.8, 41.5, 33.3, 26.1, 25.1 ppm;

LRMS *m/z* (ES+) *m/z* 263 $[M + H]^+$.

N-((5-chloro-1H-indol-3-yl)methyl)cycloheptanamine (5f). Yield: 12%

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.68 (br. s., 1H), 6.74 - 6.96 (m, 4H), 3.59 (s., 2H), 2.47 (s, 1H), 2.22 (br. s., 1H), 1.45 - 2.05 (m, 6H), 0.27 - 1.02 (m, 6H) ppm;

^{13}C NMR (101 MHz, CHLOROFORM-d) δ 133.6, 127.0, 123.6, 123.2, 121.1, 117.0, 112.8, 111.3, 57.9, 40.9, 33.5, 27.2, 23.4 ppm;

LRMS *m/z* (ES+) *m/z* 277 $[M + H]^+$.

N-((6-chloro-1H-indol-3-yl)methyl)cyclohexanamine (5g). Yield: 76%

^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.23 (br. s., 1H), 7.55 (d, J = 8.43 Hz, 1H), 7.34 (d, J = 1.64 Hz, 1H), 7.13 (d, J = 1.00 Hz, 1H), 7.10 (dd, J = 1.89, 8.44 Hz, 1H), 3.99 (s, 2H), 2.54 - 2.62 (m, 1H), 1.59 - 2.01 (m, 10H) ppm;
13C NMR (101 MHz, CHLOROFORM-d) δ 135.6, 126.9, 124.6, 121.9, 119.2, 118.6, 114.4, 110.1, 55.5, 40.6, 32.4, 25.1, 24.0 ppm;
LRMS *m/z* (ES+) *m/z* 263 $[M + H]^{+}$.

N-((6-chloro-1H-indol-3-yl)methyl)cycloheptanamine (5h). Yield 73%
^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.22 (br. s., 1H), 7.56 (d, J = 8.43 Hz, 1H), 7.35 (s, 1H), 7.16 (s, 1H), 7.10 (d, J = 8.44 Hz, 1H), 4.08 (br. s, 1H), 3.90 (s, 2H), 2.73 - 2.85 (m, 1H), 1.80 - 2.07 (m, 6H), 1.62 - 1.80 (m, 4H), 1.26 (s., 2H) ppm;
^{13}C NMR (101 MHz, CHLOROFORM-d) δ 133.6, 128.0, 127.9, 123.4, 120.3, 119.7, 111.2, 111.2, 58.7, 42.0, 34.5, 28.3, 24.4 ppm;
LRMS *m/z* (ES+) *m/z* 277 $[M + H]^{+}$.

N-((5-methyl-1H-indol-3-yl)methyl)cyclohexanamine (5i). Yield 54%
^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.16 (br. s., 1H), 7.42 (s, 1H), 7.25 (s, 1H), 7.13 (d, J = 1.00 Hz, 1H), 7.03 (t, J = 8.30 Hz, 1H), 4.01 (s, 2H), 2.59 - 2.67 (m, 1H), 2.47 (s, 3H), 2.19 (br. s, 1H), 1.73 - 1.80 (m, 2H), 1.11 - 1.33 (m, 8H) ppm;
^{13}C NMR (101 MHz, CHLOROFORM-d) δ 134.6, 128.8, 127.2, 123.7, 123.0, 118.2, 113.9, 110.9, 56.4, 41.6, 33.2, 26.1, 25.1, 21.6 ppm;
LRMS *m/z* (ES+) *m/z* 243 $[M + H]^{+}$.

N-((5-methyl-1H-indol-3-yl)methyl)cycloheptanamine (5j). Yield 72%
^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.16 (br. s., 1H), 7.42 (s, 1H), 7.25 (s, 1H), 7.21 (d, J = 1.89 Hz, 1H), 7.03 (dd, J = 1.38, 8.31 Hz, 1H), 4.01 (s, 2H), 2.84 (tt, J = 4.31, 8.91 Hz, 1H), 2.47 (s, 3H), 1.64 - 1.78 (m, 3H), 1.57 - 1.62 (m, 2H), 1.35 - 1.54 (m, 5H), 1.18 - 1.28 (m, 2H) ppm;
^{13}C NMR (101 MHz, CHLOROFORM-d) δ 134.5, 128.9, 127.3, 123.8, 120.0, 118.2, 113.6, 111.0, 58.3, 41.4, 33.9, 28.2, 24.4, 21.6 ppm;
LRMS *m/z* (ES+) *m/z* 257 $[M + H]^{+}$.

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9.APPENDIX

9.1. Abbreviations used

XDR, extensively drug resistant; MDR, multidrug resistant; TDR, totally drug resistant; RR- TB, rifampicin resistant tuberculosis; SAR, structure activity relation; MIC, minimal inhibitory concentration; MTB, mycobacterium tuberculosis; SI, selectivity index, MRC-5 cells, Medical Research Council cell strain 5., DOT, direct observed therapy; EPI, efflux pump inhibition; HIV, human immunodeficiency virus; AIDS, Acquired Immune Deficiency Syndrome; LTBI, latent TB infection; ART, Antiretroviral therapy.

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9.3. German abstract-Zusammenfassung:

Die gegenständliche Arbeit befasst sich mit den stetig wachsenden Herausforderungen der Behandlung der Tuberkulose. Die Erkrankung, ausgelöst durch das *mycobacterium tuberculosis*, welche vornehmlich die Lunge betrifft, wurde in den vergangenen Jahren von der WHO als globales Gesundheitsrisiko eingestuft, nachdem die Erkrankung Anfang der 1990er besiegt geglaubt war (WHO, 2016). Die neuerliche Gefährdung erwächst aus steigenden Fällen von multiresistenten Infektionen. Dies betrifft vor allem Rifampicin und Isoniazid, zwei der vier wichtigsten antituberkulären Arzneistoffe.

Die gegenständliche Arbeit hat sich zur Aufgabe gemacht neue Leitstrukturen, vor allem zur Behandlung von multiresistenten Tuberkuloseinfektionen zu entwerfen und zu synthetisieren. Dabei wurden vorangegangene Arbeiten, die sich mit der Modifizierung von Thioridazin (Scalacci, et al., 2017), einem Reserve Antituberkulotikum und der Verbesserung von SQ109 und BM212 (Bhakta, et al.,

2016), zwei bereits länger bekannten Strukturen mit antituberkulärer Aktivität, befasst haben, herangezogen. Aufgrund der aus diesen Arbeiten hervorgegangenen Erkenntnisse wurden für die Synthese der gegenständlichen Strukturenbibliothek *p*-Cl-phenyl und cycloaklylamin substituierte Thiaziole, beziehungsweise 3-cycloalkylaminoindole als Grundstruktur gewählt. Vor allem für die Thiazolderivate ist aufgrund vorangegangener Publikationen mit einer guten bis sehr guten antituberkulären Wirkung zu rechnen. Für die synthetisierten Indolderivate lässt sich eine gute, womöglich jedoch klinisch nicht ausreichende antituberkuläre Wirkung erwarten.
Im weiteren besitzen viele derart strukturverwandte Moleküle eine Efflux-Pumpen inhibierende Wirkung, wodurch deren Einsatz als Zusatz in der Behandlung der multiresistenten Tuberkulose durchaus denkbar ist.
Eine konkrete Aussage über die Wirksamkeit der im Rahmen dieser Arbeit synthetisierten Derivate ist jedoch erst nach Abschluss der laufenden biologischen Untersuchungen möglich.

9.4. Lebenslauf mit Schwerpunkt auf dem wissenschaftlichen Werdegang

Geboren am 28.09.1991 besuchte ich die Volksschule der Erzdiozöse Wien sowie das Gymnasium (AHS) des Schottenstiftes in Wien. Vor allem im Gymnasium lag mein Interessensschwerpunkt in naturwissenschaftlichen Fächern. Das bestätigte sich bei der Ablegung der Reifeprüfung in den Fächern Physik, Chemie und Biologie. Das Pharmaziestudium habe ich direkt nach der Matura an der Haupt Uni Wien angeschlossen.
Neben den Pflichtlehrveranstaltungen in den Laboratorien der Uni Wien habe ich habe ich in der A. Moll Apotheke, 1010 Wien gearbeitet.
Im Wintersemester 2016/17 habe ich einen fünfmonatigen Auslandsaufenthalt an dem King's College London, England im Rahmen der Verfassung meiner Diplomarbeit absolviert. Der wissenschaftliche Schwerpunkt lag dabei auf der nasschemischen Synthese und Aufreinigung von Pyrrol-, Indol- und Thiazolderivaten, sowie deren Analyse mittels NMR (nuclear magnetic resonance) und MS (Massen-Spektrometrie), als auch auf der Anstellung von Überlegungen zu möglichen strukturellen Verbesserungen der synthetisierten Produkte.

9.5. Author's rights - Urheberrechte

Ich habe mich bemüht sämtliche Inhaber der Bildrechte ausfindig zu machen und ihre Zustimmung zur Verwendung der Bilder in dieser Arbeit eingeholt. Sollte dennoch eine Urheberrechtsverletzung bekannt werden, ersuche ich um Meldung bei mir.