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**Synthesis of 4-nitrosalicylanilide derivatives with potential
antibacterial activity**

**Syntéza 4-nitrosalicylanilidových derivátů s potenciální
antibakteriální aktivitou**

(Diploma Thesis)

Mentor of Diploma thesis

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I declare that this thesis is my original copyrighted work. All literature and other resources I used while processing are listed in the bibliography and properly cited.

Hradec Králové 2013

Abstract

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Diploma thesis

Charles University in Prague, Faculty of Pharmacy in Hradec Kralove

Pharmacy

Background: The point of this diploma work was the synthesis of 4-nitrosalicylanilide derivatives, as a possible antibacterial agents against *Mycobacterium tuberculosis*.

Methods: All reactions were monitored, and the purity of products was verified by TLC in which the plates were coated with silica gel 60 F₂₅₄. They were also visualized using UV irradiation, while the column chromatography, was performed using silica gel 60.

The melting points of products were determined on a Melting Point apparatus and Büchi are uncorrected, IR spectra were recorded using ATR technique and the NMR spectra were measured in DMSO-*d*₆ or CDCl₃.

Results: The reactions between the starting compounds were successfully proceeded under the microwave reactor and the presence of chlorobenzene, trimethylamine and phosphorus trichloride.

Conclusion: Unfortunately some of the final products of these experiments were probably triethylamine salts of salicylanilides that was determined on NMR spectra.

Abstrakt

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Syntéza 4-nitrosalicylanilidových derivátů s potenciální antibakteriální aktivitou

Diplomová práce

Univerzita Karlova v Praze, Farmaceutická fakulta v Hradci Králové

Cíl: Cílem této diplomové práce byla syntéza několika 4-nitrosalicylanilidových derivátů s potenciální antibakteriální aktivitou.

Metody: Čistota produktů a průběh reakcí byly sledovány chromatografií na tenké vrstvě na hliníkových deskách firmy Merck potažených silikagelem 60F₂₅₄ zavizualizace UV zářením. Chromatografické čištění na koloně bylo provedeno na silikagelu 60.

Teploty tání připravených sloučenin byly měřeny na bodotávku Büchi a jsou uvedeny bez korekce, IČ spektra byla měřena technikou ART a NMR spektra byla měřena v CDCl₃ nebo DMSO-*d*₆.

Výsledky: Kondenzační reakce byly prováděny v mikrovlnném reaktoru. Výchozí sloučeniny se ponechaly reagovat v chlorbenzenu za přítomnosti trimethylaminu a chloridu fosforitého.

Závěr: Některé připravené salicylanilidy jsou patrně ve formě triethylamoniových solí což bylo určeno pomocí NMR spektroskopie.

Abbreviations

TB	Tuberculosis
WHO	World Health Organization
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Disease Syndrome
<i>M. tuberculosis</i>	<i>Mycobacterium tuberculosis</i>
PZA	Pyrazinamide
PZase	Pyrazinamidase
FASI	Fatty acid synthetase I (enzyme)
<i>M. smegmatis</i>	<i>Mycobacterium smegmatis</i>
C-1	Carbon number 1
C-3	Carbon number 3
dTDP-glucose	Thymidine diphosphate glucose
INH	Isoniazid
RIF	Rifampicin
EMB	Ethambutol
DOTS	Directly observed treatment short-course
PAS	<i>Para</i> -aminosalicylic acid
MDR-TB	Multidrug resistant tuberculosis
XDR-TB	Extensively drug-resistant tuberculosis
ETH	Ethionamide

Mar	Multiple antibiotic resistance
Rv1931	MarA homolog
mRNA	Messenger ribonucleic acid
C14-INH	C14 Isoniazid (C14 acyl chain)
H37Ra	Attenuated strain
UV	Ultraviolet
IR	Infra Red
ATR	Attenuated Total Reflectance
NMR	Nuclear Magnetic Resonance
DMSO-d ₆	Dimethyl sulfoxide-d ₆
CDCl ₃	Deuterated chloroform
PCl ₃	Phosphorus trichloride
NaHCO ₃	Sodium hydrocarbonate

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1. Introduction –Tuberculosis and Its Treatment

1.1 *History of Tuberculosis*

Speaking about the history of Tuberculosis (TB), it is actually believed in our days that has been present in humans' lives for some thousands of years. According to previous findings, skeletal remains can show that some prehistoric humans (4.000 BC) appear to have tuberculosis, and tubercular decay has been found in the spines of Egyptian mummies (3.000-2.400 BC)¹.

It is also a fact that during the 17th century, specific exact pathological and anatomical descriptions of tuberculosis, began actually to appear. In about 1679, Sylvius proceeded to write his Opera Medica, in which he was then the first to identify the actual tubercles as also a consistent and for the characteristic change in the lungs and other areas of all the consumptive patients. The earliest references to the infectious nature of tuberculosis also appeared in 17th century Italian medical literature².

It was also a fact that due to the variety of the symptoms existed, tuberculosis was not identified like an unified disease until the decade of 1820s and was not actually named tuberculosis until year 1839, by J.L. Schonlein. Due to existence of tuberculosis that year, it was in 1854, when Hermann Brehmer proposed actually the idea that tuberculosis was mentioned indeed as a curable disease². In advance, the introduction of the sanatorium cure provided actually the first big step toward treatment for tuberculosis. Brehmer was also a TB patient. His doctor was the one who advised him to proceed to a healthier environment and climate, so he spent some time in the Himalayas and came home cured. This specific experience moved him so as to build the first sanatorium, a place where patients could get plenty of fresh air and good nutrition. This setup mentioned as the blueprint for the subsequent development of sanatoriums accordingly¹.

It should also be said that the measures available to doctors at the time

were still modest, although there was a feeling for development in treatment of patients³. By trying to improve any social and sanitary conditions as also to proceed by ensuring some adequate nutrition, there were all that could be done to strengthen the body's defenses against TB bacteria in those days. Sanatoriums, in our days, found throughout Europe and the United States, provided a dual function. They isolated the sick, actually the source of the infection from the general population where they forced the patients to rest, actually assisting the healing process.

Although the fact that the development of sanatoriums helped in providing a haven for those healing from tuberculosis, there were scientists and doctors who continued their research in the hopes of finding a real cure for the disease. Several techniques of the tuberculosis treatment were discovered, including actually methods³:

- Artificial pneumothorax
- Radiation
- Chemotherapy.

Those methods are analyzed as follows³:

- Artificial Pneumothorax - Forlanini, who was an Italian physician, discovered actually that lung collapse had actually a positive effect on the outcome of the mentioned disease. Upon the introduction of the artificial pneumothorax and surgical techniques so as to reduce the lung volume, the active therapy for tuberculosis began. Although this was mentioned as an accomplished technique, it was discontinued after 1946 because it proved some little benefits.
- Radiation - Some further significant advances came up actually in 1895 by the time that Wilhelm Konrad von Roentgen discovered the radiation that bears his name. With this discovery, it was now possible to follow and accurately review the progress and severity of a patient's disease.

- Chemotherapy – Concerning the method of chemotherapy in TB, it is actually a fact that in the middle of the World War II, this additional breakthrough was discovered. The administering chemotherapy to some other infectious disease, making use of sulphonamide and penicillin, this had already been underway for several years. Although, these molecules turned out to be ineffective against *Mycobacterium tuberculosis*.

But except from the above, doctors and specialists proceeded by making use of antibiotics as treatment for TB. This is mentioned to the year of 1943, where Selman A. Waksman, had been working for decades so as to find a specific antibiotic that could be effective against the *Mycobacterium tuberculosis*⁴. The medicine of streptomycin purified from *Streptomyces griseus* that was first administered to a human on November 20, 1944. The results were also appeared to be extremely impressive. The disease immediately stopped its progression, the bacteria disappeared from the patient's sputum, and he was then recovered fully.

It is also mentioned that a rapid succession of anti-TB drugs was also appeared during the following years. This was something very important due to the fact that upon the streptomycin treatment, resistant mutants began actually to appear within a few months, endangering the success of antibiotic therapy. However, it was soon proven that using a combination of drugs would solve this problem¹.

But since then and despite all the drugs available today, tuberculosis is still considered to be a problem in various nations. According to World Health Organization (WHO), it is estimated that each year, about eight millions of people worldwide could develop some active tuberculosis and close to two million die, unfortunately⁴. Although the overall rate of the new tuberculosis cases has actually continued to decline in the United States since national reporting began in 1953, the annual decrease in tuberculosis cases appeared to have slowed dramatically. TB still continues to kill between two and three

millions people every year. The WHO estimates also that thirty six (36) million people will die of the tuberculosis by 2020 if this could be not controlled.

Another fact that actually plays an important role in development of TB since our days, is that mentioned that the registered number of new cases of TB worldwide could roughly be correlated upon the economic conditions. It has been found that the highest incidences are found in these countries of Africa, Asia, and Latin America comprising the lowest gross national products. According to the above, the World Health Organization estimates actually that nine million people get TB every year, of whom 95% live in developing countries. An estimated 2-3 million people die from TB every year⁵.

It should also be noted that the industrialized countries, the steady drop in TB incidence started to level off in the mid-1980s and then stagnated or even started to increase accordingly. Much of this rise could also be at least partially attributed to a high rate of immigration from countries with a high incidence of TB. It is also important that a great influence in the rising TB trend is concerned to HIV infection. Appropriate chances are that only one out of ten immune-competent people infected with *M. tuberculosis* will fall sick in their lifetimes with active TB, but among those with HIV, one in ten per year will develop active TB, while one in two or three tuberculin test positive AIDS patients will develop active TB²⁶. In some industrialized countries, this is mentioned as a tragedy for the patients involved, but it these cases make up only a small minority of TB cases. In developing countries, the impact of HIV infection on the TB situation, especially in the 20-35 year age group, is of great and increasing concern.

It has also to be said that one final factor which contributes to the resurgence of TB that is the emergence of multi-drug resistance. The drug resistance in the TB occurs therefore, as a result of tubercle bacillus mutations. These mutations are not dependent upon the presence of the drug¹⁷. Exposed to a case of single effective anti-TB medication, the aspect of predominant bacilli, sensitive to that drug, are killed; the few drug resistant mutants, likely to be present if the bacterial population is large, will, multiply

freely. Since it is very unlikely that a single bacillus will spontaneously mutate to resistance to more than one drug, giving multiple effective drugs simultaneously will inhibit the multiplication of these resistant mutants. This is why it is absolutely essential to treat TB patients with the recommended four-drug regimen of isoniazid, rifampin, pyrazinamide, and ethambutol or streptomycin⁵.

Finally it is actually mentioned that although there is a number of wealthy industrialized countries upon good public health care systems that can be expected so as to keep TB under control, in some of the developing world a catastrophe awaits. It is also important that support could be given to research efforts devoted to developing an effective TB vaccine, shortening actually the amount demanded for the time required so as to ascertain drug sensitivities, improving also the diagnosis of TB and creating by far some new, highly effective anti-TB medications. Without any support for such efforts, people run the risk of losing the battle against TB⁵.

1.2 Pathophysiology of Tuberculosis

Mentioned to the pathophysiology of Tuberculosis (TB) in ourdays, it has actually been mentioned that sometimes is concerned to be under recognized; People start first upon the way of how they would be able to obtain a definitive diagnosis of tuberculosis. In such cases, it is mentioned that tuberculosis has actually reemerged as a major health concern in recent years³.

Only in the United States, it has been noted that approximately 14.000 cases of tuberculosis were reported in 2011, a 3.2% decline from the previous year of 2010 although, about 20 states and the District of Columbia had higher rates of TB¹. It is also mentioned that the prevalence of tuberculosis is

continuing to increase because of the increased number of patients infected with human immunodeficiency virus, bacterial resistance to medications, increased international travel and immigration from countries with high prevalence, and the growing numbers of the homeless and drug abusers³.

It is also a fact that the vulnerability of the hospitalized patients to the disease of tuberculosis is often concerned to be under recognized due to the infection which is habitually considered a disease of the community accordingly. Most hospitalized patients are in a suboptimal immune state, particularly in intensive care units, making exposure to tuberculosis even more serious than in the community. By understanding of the causative organism, pathophysiology, transmission, and diagnostics of tuberculosis and the clinical manifestations in patients, critical care are better prepared to recognize infection, prevent transmission, and treat this increasingly common disease².

Tuberculosis is mentioned as an infection caused by the rod-shaped, non-spore-forming, aerobic bacterium *Mycobacterium tuberculosis*³. It is provided that mycobacteria typically measure 0.5 μm by 3 μm , are classified as acid-fast bacilli, and have a unique cell wall structure crucial to their survival. The well-developed cell wall contains a considerable amount of a fatty acid, mycolic acid, covalently attached to the underlying peptidoglycan-bound polysaccharide arabinogalactan, providing an extraordinary lipid barrier.

This specific barrier is concerned to be responsible for a number of the medically challenging physiological characteristics of tuberculosis, including resistance to antibiotics and host defense mechanisms⁷. The composition and quantity of the cell wall components affect the bacteria's virulence and growth rate⁵. It is a fact that the peptidoglycan polymer can confer cell wall rigidity and it is just external to the bacterial cell membrane, another contributor to the permeability barrier of mycobacteria. In advance, an important component of the cell wall is mentioned the lipoarabinomannan, a carbohydrate structural antigen on the outside of the organism that is immunogenic and facilitates the

survival of mycobacteria within macrophages⁴. As to the cell wall, this is mentioned as the key to the survival of mycobacteria, and a more complete understanding of the biosynthetic pathways and gene functions and the development of antibiotics to prevent formation of the cell wall are areas of great interest⁵.

Based on the above, it is also mentioned that once inhaled, the infectious droplets can settle throughout the airways. The majority of the bacilli are trapped in the upper parts of the airways where the mucus-secreting goblet cells exist⁵. Then the mucus produced, it can catch some foreign substances as the cilia on the surface of the cells can beat the mucus and its entrapped particles upward for removal⁴. This specific system provides the body with an initial physical defense that prevents infection in most persons exposed to tuberculosis².

It is also noticed that the bacteria mentioned in droplets that bypass the mucociliary system and reach the alveoli, they are quickly surrounded and engulfed by alveolar macrophages, the most abundant immune effector cells present in alveolar spaces¹. Those macrophages, the next line of host defense, are actually part of the innate immune system and provide an opportunity for the body to destroy the invading mycobacteria and prevent infection⁵.

The macrophages are readily available to phagocytic cells which could combat many pathogens without requiring previous exposure to the pathogens. Various mechanisms and macrophage receptors are also involved in the uptake of the mycobacteria⁴. This mycobacterial lipoarabinomannan is mentioned as a key ligand for a macrophage receptor. The complement system also plays a role in the phagocytosis of the bacteria. It is also mentioned that the complement protein C3 binds to the cell wall and enhances recognition of the mycobacteria by macrophages. The procedure of opsonization by C3 is rapid, even in the air spaces of a host with no previous exposure to *M. tuberculosis*³. It is also a fact that the subsequent phagocytosis by macrophages initiates a cascade of events that results in

either successful control of the infection, followed by latent tuberculosis, or progression to active disease, called primary progressive tuberculosis²¹. The outcome is mentioned essentially to be determined by the quality of the host defenses and the balance that occurs between host defenses and the invading mycobacteria⁸.

In the next stage and after being ingested by macrophages, the mycobacteria continue to multiply slowly, with bacterial cell division occurring every 25 to 32 hours⁵. Regardless of the fact if the infection can become controlled or progresses, any initial development can involve the production of proteolytic enzymes and cytokines by macrophages in an attempt to degrade the bacteria⁴. The released cytokines can attract the T lymphocytes to the site where the cells that constitute cell-mediated immunity.

For those patients upon intact cell-mediated immunity, the next defensive step is formation of granulomas around the *M. tuberculosis* organisms⁵. These nodular-type lesions form from an accumulation of activated T lymphocytes and macrophages, which creates a micro-environment that limits replication and the spread of the mycobacteria⁵. This environment destroys macrophages and produces early solid necrosis at the centre of the lesion; however, the bacilli are able to adapt to survive.

It is actually a fact that *M. tuberculosis* organisms can also change their phenotypic expression, like the protein regulation so as to enhance survival². By 2 or 3 weeks, the characterized as necrotic environment resembles soft cheese, often referred to caseous necrosis, and is characterized by low oxygen levels, low pH, and limited nutrients. This specific condition restricts further growth and establishes latency. In advance some lesions in persons with an adequate immune system generally undergo fibrosis and calcification, successfully controlling the infection so that the bacilli are contained in the dormant, healed lesions⁵. Those lesions in persons with less effective immune systems progress to primary progressive tuberculosis¹.

Finally it should be said that for less immunocompetent persons,

granuloma formation is initiated yet ultimately is unsuccessful in containing the bacilli⁴. It is also a fact that the necrotic tissue undergoes liquefaction, and the fibrous wall loses structural integrity. The semiliquid necrotic material can then drain into a bronchus or nearby blood vessel, leaving an air-filled cavity at the original site. In patients infected with *M. tuberculosis*, droplets can be coughed up from the bronchus and infect other persons. If any discharge into a vessel occurs, then occurrence of extrapulmonary tuberculosis is likely to happen⁵. Bacilli can also drain into the lymphatic system and collect in the tracheobronchial lymph nodes of the affected lung, where the organisms can form new caseous granulomas³.

1.3 Appropriate Drugs for Tuberculosis in Nowadays

1.3.1 Drugs of 1st Line

- Rifampicin

Rifampicin is an antibiotic used to treat infections, including tuberculosis. It is usually prescribed as one of a number of medicines to treat the infection. You may have been prescribed it for this reason. Alternatively, you may have been prescribed it to protect you from a serious infection caused by certain bacteria. Rifampicin works by stopping the bacteria causing the infection from growing and multiplying².

Tuberculosis is a bacterial infection which is mostly found in the lungs but which can affect any part of your body. TB is treatable with a course of medicines which usually last six months. Several medicines are given together to treat TB. There are some brands of tablets available which contain rifampicin in combination with one or more of the other medicines used to treat TB.

- **Isoniazid**

Isoniazid is used to treat tuberculosis or prevent its return (reactivation). It may be given alone, or in combination with other medicines, to treat TB or to prevent its return (reactivation). This medicine may also be used for other problems as determined by patients doctor¹. This medicine may cause some serious side effects, including damage to the liver. Liver damage is more likely to occur in patients over 50 years of age. Patients and their doctor should talk about the good this medicine will do, as well as the risks of taking it³.

If patients are being treated for active tuberculosis (TB): To help clear up patients' TB infection completely, they must keep taking this medicine for the full time of treatment, even if patients begin to feel better. This is very important. It is also important that they do not miss any doses. Isoniazid is available only with patients' doctor's prescription. This product is available in the following dosage forms:

- Syrup
- Tablet
- Solution

- **Ethambutol**

Ethambutol is an antibiotic that is used to treat tuberculosis. It works by stopping the bacteria that cause this disease from growing and increasing in numbers. There are two stages in the treatment for tuberculosis. In the first two months treatment is aimed at killing as many bacteria as possible⁹. Therefore several anti-TB drugs with different mechanisms of action are used at the same time (usually rifampicin, pyrazinamide, isoniazid and ethambutol). After this time some of the medicines are stopped and the others (usually rifampicin and isoniazid) are continued for a further four months to kill any

remaining bacteria. Ethambutol is used in the initial phase of treatment only².

The bacteria that cause TB are difficult to treat. By using several medicines at the same time, the bacteria can be targeted in different ways and hence treatment is more likely to be effective than using a single medicine. In addition, using different medicines together makes it less likely that bacteria will develop resistance to the treatment. Ethambutol tablets are usually taken once daily with or without food. The tablets should be swallowed with a drink of water. Take ethambutol exactly as directed. Patients should follow the directions printed on the medicine label carefully¹.

- **Pyrazinamide**

Pyrazinamide (PZA) is an antimicrobial agent that is most commonly used for treatment of active tuberculosis during the initial phase of therapy (generally the first two months of treatment), in combination with other agents. The spectrum of PZA is relatively narrow; it demonstrates clinically significant antibacterial activity only against *Mycobacterium tuberculosis* and *M. africanum*⁵. Issues related to the clinical use of pyrazinamide will be reviewed here. Issues related to treatment of latent and active TB are discussed separately.

The parent compound is metabolized via pyrazinamidase (PZase) to pyrazinoic acid; pyrazinoic acid is the active form of the drug⁴. The mechanism of action for PZA is not known. PZA and its analog, 5-chloro-PZA, may inhibit the fatty acid synthetase I (FASI) enzyme of *M. tuberculosis*³. PZA is generally considered to be a bacteriostatic agent.

PZA thought to be more active at an acidic pH (eg, within macrophages) and against dormant or semidormant microorganisms, although the role of PZA against intracellular organisms remains uncertain. In one study, pyrazinoic acid remained outside of *M. tuberculosis* cells at a neutral or alkaline pH but accumulated within cells at an acidic pH. In the same study, *M. smegmatis* (which is not susceptible to PZA) was found to

convert PZA to pyrazinoic acid but due to an active efflux mechanism did to accumulate the metabolite, even at an acidic pH. Other mycobacterial strains appear to have natural resistance to PZA due to lack of PZase activity or absence of transport mechanisms to take up the drug².

When used as part of combination therapy, PZA appears to accelerate the sterilizing effect of isoniazid and rifampin⁵. In selected populations this has enabled reduction in the duration of treatment infection due to susceptible *M. tuberculosis* isolates from nine to six months.

- **Streptomycin**

Streptomycin is an aminocyclitol-aminoglycoside antibiotic produced by *Streptomyces griseus*. Streptomycin consists of aminocyclitol (streptidine), 6-deoxyhexose (streptose), and *N*-methyl-L-glucosamine moieties, which are formed by independent biosynthetic pathways. All of the moieties are derived from D-glucose. The streptidine moiety is synthesized via myo-inositol, which is then oxidized at C-1 and transaminated to give scyllo-inosamine. After phosphorylation, the compound is transaminated by arginine. The same procedure is repeated at the C-3 position. The streptose moiety is made from D-glucose via a dTDP-glucose pathway. The exact biosynthetic route for the *N*-methyl-L-glucosamine moiety is unknown, though the biosynthetic gene cluster has been proposed³.

The four mainstream first-line drugs for treating active tuberculosis as mentioned above, are isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA). The combination of these four anti-tuberculosis drugs constitutes the basis of the directly observed treatment short-course (DOTS) strategy internationally recommended by WHO to treat smear positive TB patients infected with drug-susceptible strains of *M. tuberculosis*. The DOTS strategy was developed in order to limit the development of drug-resistant *M. tuberculosis* strains⁸.

A proper management of the DOTS strategy consists of administration of INH, RIF, EMB, and PZA for two months, followed by the use of INH and RIF for an additional four months for a total duration of six months. During the two first months, in some countries and in some situations, EMB is sometime substituted by streptomycin, which is considered a first-line TB drug as well, but not preferred when EMB is available. Application of the DOTS strategy is effective even in cases when TB patients are infected with strains of *M. tuberculosis* resistant to one of the four mainstream TB drugs⁸.

1.3.2 Drugs of 2nd Line

Ciprofloxacin is used to treat or prevent certain infections caused by bacteria. Ciprofloxacin is also used to treat or prevent anthrax (a serious infection that may be spread on purpose as part of a bioterror attack) in people who may have been exposed to anthrax germs in the air. Ciprofloxacin extended-release (long-acting) tablets are used only to treat certain types of urinary tract infections. Ciprofloxacin is in a class of antibiotics called fluoroquinolones. It works by killing bacteria that cause infections. Antibiotics will not work for colds, flu, or other viral infections⁴.

Ciprofloxacin comes as a tablet, a suspension (liquid), and an extended-release tablet to take by mouth. The tablet and suspension are usually taken twice a day, in the morning and evening, with or without food. The extended-release tablets are usually taken once a day. Cipro XR brand extended-release tablets may be taken with or without food.

Proquin XR brand extended-release tablets should be taken with a main meal of the day, preferably the evening meal. Take ciprofloxacin at around the same time(s) every day. The length of your treatment depends on the type of infection you have. Patients' doctor will tell them how long to take ciprofloxacin. They should follow the directions on their prescription label carefully, and ask their doctor or pharmacist to explain any part they do not understand. They have to take ciprofloxacin exactly as directed. They should not take more or less of it or take it more often than prescribed by their

doctor¹.

One brand or type of ciprofloxacin cannot be substituted for another. They have to be sure that they receive only the brand or type of ciprofloxacin that was prescribed by their doctor. They also should not take ciprofloxacin with dairy products or calcium-fortified juices alone. However, they may take ciprofloxacin with a meal that includes these foods or drinks. They should swallow the tablets whole; do not split, crush, or chew them. If they cannot swallow tablets whole, tell their doctor.

If patients are taking the suspension, they should shake the bottle very well for 15 seconds before each use to mix the medication evenly. They should swallow the correct dose without chewing the granules in the suspension. They have to close the bottle completely after each use. Do not give the suspension to a patient through a feeding tube. Lastly they should begin feeling better during the first few days of their treatment with ciprofloxacin. If their symptoms do not improve or if they get worse, they have to call their doctor. If they are being treated for a urinary tract infection, they have to call their doctor if patients develop fever or back pain during or after their treatment. These symptoms may be signs that their infection is worsening².

- **Clarithromycin**

Clarithromycin is used to treat certain types of bacterial infections. It works by killing certain types of bacteria. Other information about Clarithromycin³:

- *This medicine is a powder.* Water will be added to the medicine to make it into a liquid for patients to take. This is usually done by the pharmacy at the time of dispensing. Dispose of the made-up solution 14 days after the day that water was added. It is a good idea to make a note of the date that water was added so you will know when to dispose of Clarithromycin

- They do not share their medicine with other people. It may not be suitable for them and may harm them.
- The pharmacy label on their medicine tells patients how much medicine they should take. It also tells them how often they should take their medicine. This is the dose that patients and their prescriber have agreed they should take. They should not change the dose of their medicine unless patients are told to do so by their prescriber.
- If patients feel that the medicine is making them unwell or they do not think it is working, then talk to their prescriber.
- Clarithromycin is not suitable for everyone and some people should never use it. Other people should only use it with special care. It is important that the person prescribing this medicine knows their full medical history.

Their prescriber may only prescribe this medicine with special care or may not prescribe it at all if patients⁴:

- are allergic or sensitive to or have had a bad reaction to macrolides in the past
- are allergic or sensitive to or have had a reaction to any of the ingredients in the medicine
- have a slow heart rate
- have kidney problems
- have metabolic problems
- have myasthenia gravis
- have or have had heart problems
- have or have had liver problems

Furthermore the prescriber may only prescribe this medicine with special care or may not prescribe it at all for a child under the age of six months or weighs less than eight Kg. As part of the process of assessing suitability to take this medicine a prescriber may also arrange tests to

determine whether or not the medicine is suitable and whether it must be prescribed with extra care¹. Over time it is possible that Clarithromycin can become unsuitable for some people, or they may become unsuitable for it. If at any time it appears that Clarithromycin has become unsuitable, it is important that the prescriber is contacted immediately.

- **Cycloserine**

Cycloserine is an antibiotic that is used to treat tuberculosis (TB)¹⁰. It works by killing the bacteria that cause this disease. Cycloserine stops the tuberculosis bacteria from making cell walls correctly. The cell walls of bacteria are vital for their survival. They keep unwanted substances from entering their cells and stop the contents of their cells from leaking out. By weakening their cell walls, cycloserine kills the bacteria. Tuberculosis bacteria are difficult to eliminate and can become resistant to the medicines that are used to treat this infection. Cycloserine is used when the bacteria have become resistant to other medicines that are usually prescribed to treat TB.

Cycloserine will be given in combination with other medications that target the bacteria in different ways. This helps to make it more difficult for the bacteria to develop resistance to the medicines. It is used for the following cases¹:

- Treatment of tuberculosis that is resistant to first-line medicines (isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol), or when these medicines cause unacceptable side effects. Cycloserine is used in combination with other anti-tuberculous medicines.
- Cycloserine capsules should be swallowed with a drink of water. They can be taken either with or without food.
- Cycloserine capsules are usually taken twice a day (morning and evening, every 12 hours). The number of capsules to take will vary from person to person. It is important to follow the instructions given by

their doctor. These will also be printed on the dispensing label that your pharmacist has put on the packet of medicine.

- Patients have to try to always take their doses at the same times. This will help them remember to take them.
- If patients forget to take a dose, take it as soon as they remember, unless it is almost time to take their next dose. In this case, they should skip the missed dose and take their next dose when it is due.
- They should not take a double dose to make up for a missed dose.

- **Capreomycin**

Capreomycin is an injectable broad-spectrum antibiotic used in the therapy of drug-resistant tuberculosis as a second line agent, always in combination with other antituberculosis drugs¹⁰. Capreomycin has not been linked to cases of clinically apparent liver disease⁴. Capreomycin is a polypeptide antibiotic initially isolated from the bacterium, *Streptomyces capreolus*. Capreomycin is believed to act by binding to bacterial ribosomes and inhibiting protein synthesis. Capreomycin is similar to viomycin and is considered bacterostatic rather than bacterocidal. While structurally different, capreomycin has similar antibacterial activity and side effects to the aminoglycosides (streptomycin, kanamycin, amikacin)².

Capreomycin is currently used only as a secondary agent in the treatment of active tuberculosis, always in combination with other antituberculosis agents such as isoniazid, ethambutol, pyrazinamide and/or rifampin (but not streptomycin or the aminoglycosides) and usually for multi-drug resistant mycobacterial infections. Capreomycin was approved for use in the United States in 1973 but is currently rarely used, streptomycin or a modern aminoglycoside being used in its place. Capreomycin is available in vials for intramuscular or intravenous injection under the trade name of Capastat. The typical dose is 15 mg/kg/day (maximum of 1 gram per day)

intramuscularly for 2 to 4 months. The dose must be modified based upon renal function⁵.

Intramuscular therapy with capreomycin has not been definitely linked to liver injury, either in the form of asymptomatic elevations in serum enzymes or of clinically apparent liver injury. However, the agent is rarely used and is always used in combination with other antituberculosis medications, many of which are well known to cause liver injury. Nevertheless, in the few studies done, capreomycin has not been associated with an increase in the rate of serum enzyme elevations or in episodes of acute liver injury above or beyond what was reported in the comparator arm¹.

Capreomycin is excreted largely unchanged in the urine. Its lack of hepatic metabolism and known renal toxicity may account for its lack of hepatotoxicity. The majority of toxicities of capreomycin resemble those of streptomycin and the aminoglycosides and includes oto- and nephrotoxicity⁵. First line medications used in the therapy of tuberculosis in the United States include isoniazid, pyrazinamide, ethambutol and the rifamycins including rifampin, rifabutin and rifapentine. Second line medications include streptomycin, capreomycin, cycloserine, ethionamide, fluoroquinolones such as levofloxacin, and moxifloxacin, aminoglycosides such as amikacin and *para*-aminosalicylic acid (PAS)¹.

It should also be mentioned that while the DOTS strategy has shown to be effective at curing TB cases and limiting the occurrence of drug-resistant *M. tuberculosis* strains in countries where the program is rigorously managed, nevertheless, cases of drug-resistant TB are still recorded. Conventionally, *M. tuberculosis* strains that are resistant to at least INH and RIF are termed multidrug resistant *M. tuberculosis* (MDR-TB)⁸.

Anti-tuberculosis compounds used to treat MDR-TB cases are called second line anti-tuberculosis drugs. They are also commonly used to treat immune-compromised patients such as HIV patients who are infected with

atypical mycobacterial species. Though effective against *M. tuberculosis* at different degrees, second line TB drugs have the disadvantage of exhibiting relatively strong side effects in comparison with their first-line drugs counterparts⁸.

Treatment of MDR-TB strains is more complex and requires characterization of strains in order to adapt the treatment to each patient. In MDR-TB cases, a 24-month regimen is usually recommended with the use of one or several second-line drugs. The TB pandemic is even more complex today because of emergence of *M. tuberculosis* strains resistant to not only first-line anti-tuberculosis drugs, but also to some second-line drugs. These TB strains resistant to both first-line and second-line drugs are named extensively drug-resistant tuberculosis (XDR-TB).

Aminoglycosides are able to be used in treating MDR-TB comprise kanamycin and its derivative, amikacin. Both kanamycin and amikacin are administered parenterally¹¹. Capreomycin, another injectable second-line anti-tuberculosis drug is a macrocyclic polypeptide agent used to treat MDR-TB patients. It has a different chemical structure from that of kanamycin and amikacin, but share with these two aminoglycosides, the same mechanism of action, leading to the conclusion that acquisition of resistance to one drug may also cause a cross-resistance to other drugs⁸.

Nevertheless, this phenomenon could also be extremely variable from one strain to another. In spite of its bacteriostatic activity on *M. tuberculosis*, D-cycloserine has not been very much used in traditional TB treatment because of its neurotoxic effects on TB patients. It is only used in combination with other drugs in case of MDR-TB infection. D-cycloserine is a structural analog of D-alanine, an essential amino acid in bacterial cell wall synthesis. Several fluoroquinolones exhibits an anti-mycobacterial activity *in vitro*, but only a small fraction including ciprofloxacin, levofloxacin, ofloxacin, and sparfloxacin has shown to be clinically active against *M. tuberculosis*⁸.

Ciprofloxacin and ofloxacin have been used with success against TB cases recently¹², but the emergence of drug resistance casting doubt as to whether fluoroquinolones should be used in TB treatment. What is more, fluoroquinolones affect the growth of *M.tuberculosis* by targeting *gyrA* and *gyrB*, two genes encoding DNA gyrases. Resistance to fluoroquinolones is caused by mutations in mycobacterial genes *gyrA* and *gyrB*, *para*-aminosalicylic acid (PAS) was introduced as TB treatment option by the end of 1940s⁸.

PAS was extensively used worldwide in the treatment of TB until about 2000 despite gastric side effects. Treatment of TB using PAS requires relatively large doses of drug because of its high level of excretion. PAS has a bacteriostatic effect of *M. tuberculosis*⁴. Today, PAS tends to be used for treating MDR-TB patients (Peloquin) as better drugs exist to treat susceptible *M. tuberculosis* strains. Thioamides compounds including ethionamide (ETH) and prothionamide are recommended and in fact very commonly used in the treatment of MDR-TB cases. Although the specific mechanisms of action of thioamides compounds have not been fully elucidated, these antituberculosis drugs appear to inhibit synthesis of mycolic acids⁸.

1.4 Role of Salicylates in Tuberculosis

Salicylate is a ubiquitous molecule that is involved in systemic acquired disease resistance in plants¹³. Salicylate is the active component of aspirin, which is one of the most popular medicines used in the prevention and treatment of a wide variety of disease conditions. During the analysis of the effect of organic acids on the oxidative metabolism of the tubercle bacillus in the 1940s, Bernheim found that salicylate increased the oxygen consumption of the bacilli, whereas structurally related benzoic acid had a much weaker effect¹³.

Based on this observation, Lehmann and colleagues identified the salicylate analogue *p*-aminosalicylic acid (PAS) as an effective

antituberculosis (anti-TB) agent (now used as a second-line TB drug)⁵, even though the mode of action of PAS is probably not directly related to the effect of salicylate on tubercle bacilli.

Salicylates were first described to induce a multiple antibiotic resistance (Mar) phenotype in *Escherichia coli*¹³, and it is now known to induce antibiotic resistance in a variety of bacterial species¹³. In the *E. coli* system, salicylate induces antibiotic resistance by binding to MarR and activating the transcription of *marA* and *marB*. MarA and MarB regulate a range of genes that confer a Mar phenotype, including down-regulation of OmpF porin expression via *micF* antisense RNA to limit the entry of antibiotics¹³ and switching on of efflux pumps such as AcrAB to more effectively extrude antibiotics from the cells¹³.

However, salicylate also induces antibiotic resistance through a Mar-independent pathway, since a Mar deletion strain still showed resistance in the presence of salicylate¹³. In gram-positive bacteria such as *Staphylococcus aureus*, salicylate induces resistance to antibiotics such as fluoroquinolone, although the mechanism involved is unknown. One possible mechanism is that *M. tuberculosis* may have a Mar-like regulatory mechanism as in *E. coli*.

Over expression of the *E. coli* marA on a multicopy plasmid in the fast growing organism *M. smegmatis* mediates resistance to multiple antimycobacterial agents, indicating the presence of a mar-like regulatory system in this organism¹³. However, MarA over expression was not performed with *M. tuberculosis* in that study. A MarA homolog (Rv1931) is present in the *M. tuberculosis* genome; however, the degree of homology of Rv1931 to MarA is quite low.

Preliminary studies showed that the Rv1931 mRNA was not induced by salicylate (data not shown), indicating that Rv1931 is unlikely to be responsible for the salicylate-induced drug resistance in *M. tuberculosis*. On the other hand, salicylate may reduce the permeability of the mycobacterial cell membrane as a mechanism for the salicylate induced drug resistance in *M. tuberculosis*.

A preliminary study using C14-INH has suggested that the uptake of INH by *M. tuberculosis* H37Ra is reduced in the presence of salicylate (data not shown). The possibility of an involvement of efflux pumps in salicylate-induced resistance in *M. tuberculosis* remains to be tested. Further studies are needed to determine the precise mechanism of the salicylate-induced drug resistance in *M. tuberculosis*. Because salicylate is widely used in the prevention and treatment of diverse disease conditions at concentrations (up to 2 μ M)¹³ that are known to induce resistance to various drugs in *M. tuberculosis* in vitro, it will be of interest to assess the effect of salicylate on the treatment of TB in animal models and in humans.

2. Experimental part

The aim of the thesis

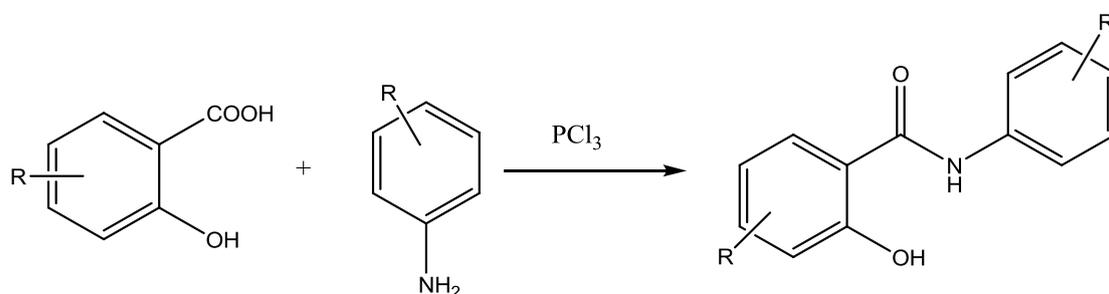
General methods

The reactions were monitored, and the purity of products was verified by thin layer chromatography in which the plates were coated with 0.2 mm silica gel 60 F₂₅₄ (Merck, Prague, Czech Republic) and visualized using UV irradiation (254 and 366 nm). Column chromatography was performed using silica gel 60 with a particle size of 0.063-0.2 mm (Fluka, Prague, Czech Republic).

The melting points of products were determined on a Melting Point B-540 apparatus (Büchi Labortechnik AG, Flawil, Switzerland) in open capillaries and are uncorrected. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) over the range of 400-4000 cm⁻¹ using ATR technique. The NMR spectra were measured in DMSO-*d*₆ or CDCl₃ solutions at ambient temperature on a Varian Mercury Vxbb 300 (300 MHz for ¹H and 75.5 MHz for ¹³C, Varian Comp. Palo Alto, CA, USA) and Varian Mercury (500 MHz for ¹H and 125 MHz for ¹³C, Varian Comp. Palo Alto, CA, USA).

Salicylanilide preparation

Reaction scheme:



General procedure:

Substituted salicylic acid (0.01 mol) was dissolved in 50 ml of chlorobenzene, 0.01 mol of substituted aniline was added. The mixture was stirred 10 minutes and after that 0.435 ml (0,005 mol) of PCl_3 was added drop by drop.

The reaction mixture was boiled under the reflux condenser in the microwave reactor for 20 minutes at 120 °C at 450 W.

After one day of standing, the precipitate was filtered off and recrystallized.

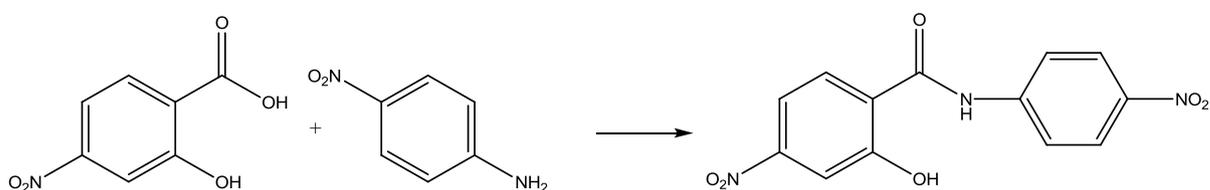
Experiment No. 1 (St 1)

Compound: 2-Hydroxy-4-nitro-*N*-(4-nitrophenyl)benzamide

Chemical Formula: C₁₃H₉N₃O₆

Molecular Weight: 303.23

Reaction scheme:



Contents:

- 1.8437 g of 2-hydroxy-4-nitrobenzoic acid
- 1.3928 g of 4-nitroaniline
- 50 ml of chlorobenzene
- 0.435 ml of PCl₃
- 1.2 ml of triethylamine

Procedure:

The mixture of the above contents was boiled in the microwave reactor at 120 °C and 400 watt for 20 minutes. After that, 30 ml of NaHCO₃ were added in order to form precipitation and this precipitate was filtrated.

This precipitate, which was named St 1(1), was recrystallized five times to obtain the final product.

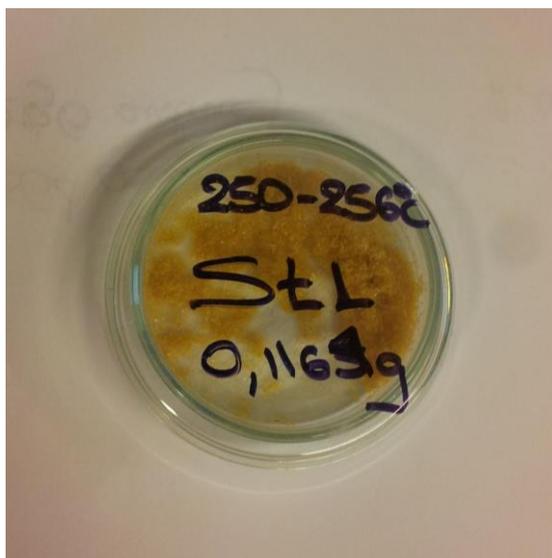
Weight of product : 0.1169 gr

m.p. :250-256 °C

^1H NMR (500 MHz, DMSO) δ 13.03 (1H, bs, NH), 7.93 (1H, d overlapped, $J=8.6$ Hz, H6), 7.93-7.89 (2H, m, AA', BB', H3', H5'), 7.70-7.65 (2H, m, AA', BB', H2', H6'), 7.50 (1H, d, $J=2.4$ Hz, H3), 7.36 (1H, dd, $J=8.6$ Hz, $J=2.4$ Hz, H5)

IR v (CONH-) : 1681 cm^{-1}

According to IR and NMR results the product from this experiment had some impurities.



Compound was found in the database Reaxys, without any characteristics, the Literature:¹⁴ which presents the compound was not on-line accessible

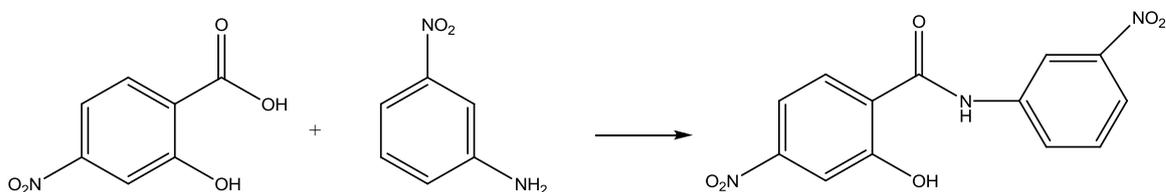
Experiment No. 2 (St 2)

Compound: 2-Hydroxy-4-nitro-*N*-(3-nitrophenyl)benzamide

Chemical Formula: C₁₃H₉N₃O₆

Molecular Weight: 303.23

Reaction scheme:



Contents:

- 1.8391 g of 2-hydroxy-4-nitrobenzoic acid
- 1.3806 g of 3-nitroaniline
- 50 ml of chlorobenzene
- 0.435 ml of PCl₃
- 1.2 ml of triethylamine

Procedure:

The mixture of the above contents was boiled in the microwave reactor at 120 °C and 400 watt for 20 minutes. After that, 30 ml of NaHCO₃ were added in order to form precipitation and this precipitate was filtrated.

This precipitate, which was named St 2(1), was mixed with diluted HCl (5ml of concentrated HCl +10 ml of distilled H₂O) and let it stirred for 15 minutes.

Then, it was filtered off and washed with distilled water. In addition, 5 ml of NaHCO_3 and small amount of water were used in order to wash away the impurities of the solution.

Finally, it was dried, recrystallized and filtered off twice to obtain the product.

Weight of product : 0.5083 gr

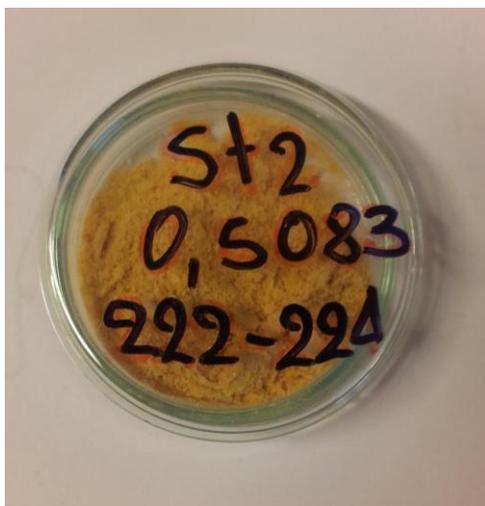
m.p. :222-224 °C

% yield= 3.61%

^1H NMR (300 MHz, DMSO) δ 11.69 (1H, bs, NH), 10.84 (1H, bs, OH), 8.76 (1H, t, $J=2.1$ Hz, H2'), 8.08-7.94 (2H, m, H4', H6'), 7.91 (1H, d, $J=8.2$ Hz, H6), 7.77 (1H, d, $J=2.1$ Hz, H3), 7.76 (1H, dd, $J=8.2$ Hz, $J=2.1$ Hz, H5), 7.65 (1H, t, $J=8.2$ Hz, H5')

^{13}C NMR (75 MHz, DMSO) δ 164.6, 156.7, 149.8, 148.2, 138.7, 131.1, 130.4, 127.4, 126.3, 118.8, 114.4, 113.9, 111.4

IR ν (CONH-) : 1641 cm^{-1}



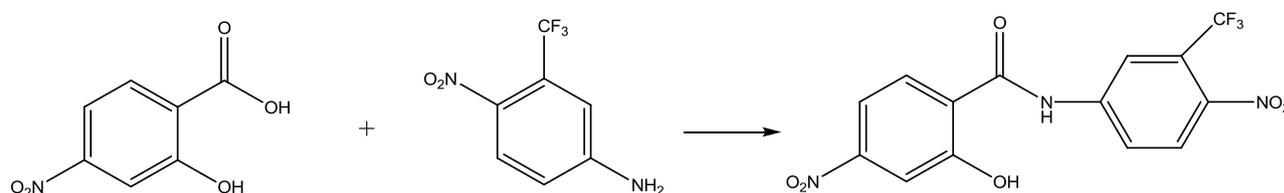
Experiment No. 3 (St 3)

Compound: 2-Hydroxy-4-nitro-*N*-(4-nitro-3-(trifluoromethyl)phenyl)benzamide

Chemical Formula: C₁₄H₈F₃N₃O₆

Molecular Weight: 371.23

Reaction scheme:



Contents:

- 1.8318 g of 2-hydroxy-4-nitrobenzoic acid
- 2.0742 g of 3-trifluoromethyl-4-nitroaniline
- 50 ml of chlorobenzene
- 0.435 ml of PCl₃
- 1.2 ml of triethylamine

Procedure:

The mixture of the above contents was boiled in the microwave reactor at 120 °C and 400 watt for 20 minutes. After that, 30 ml of NaHCO₃ were added in order to form precipitation and this precipitate was filtrated.

This precipitate, named St 3(1), was mixed with ethylacetate until completely dissolved and filtrated.

The filtrate was evaporated till dryness and then recrystallized in ethanol twice to obtain the final product.

Weight of product : 0.4446 g

m.p. :231-234 °C

% yield= 15.79%

^1H NMR (500 MHz, DMSO) δ 13.73 (1H, bs), 8.43 (1H, d, $J=2.0$ Hz, H2'), 8.19 (1H, d, $J=8.8$ Hz, H5'), 8.06 (1H, dd, $J=8.8$ Hz, $J=2.0$ Hz, H6'), 7.89 (1H, d, $J=8.3$ Hz, H6), 7.47 (1H, d, $J=2.4$ Hz, H3), 7.35 (1H, dd, $J=8.3$ Hz, $J=2.4$ Hz, H5)

^{13}C NMR (125 MHz, DMSO) δ 165.8, 164.0, 150.5, 144.4, 141.3, 131.0, 127.9, 125.2, 123.3 (q, $J=32.4$ Hz), 122.3 (q, $J=272.8$ Hz), 121.2, 118.0 (q, $J=6.7$ Hz), 113.5, 108.7

IR ν (CONH-) : 1650 cm^{-1}



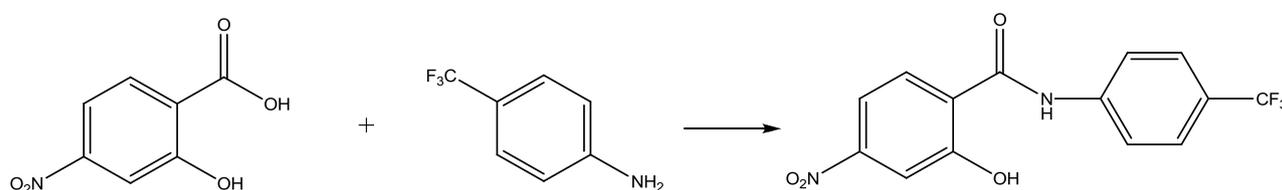
Experiment No. 4 (St 4)

Compound: 2-Hydroxy-4-nitro-*N*-(4-(trifluoromethyl)phenyl)benzamide

Chemical Formula: C₁₄H₉F₃N₂O₄

Molecular Weight: 326.23

Reaction scheme:



Contents:

- 1.8379 g of 2-hydroxy-4-nitrobenzoic acid
- 1.6160 g of 4-trifluoromethylaniline
- 50 ml of chlorobenzene
- 0.435 ml of PCl₃
- 1.2 ml of triethylamine

Procedure:

The mixture of the above contents was boiled in the microwave reactor at 120 °C and 400 watt for 20 minutes. After that, 25 ml of NaHCO₃ were added in order to form precipitation and this precipitate was filtrated.

This precipitate that was collected from this filtration was recrystallized in ethanol and filtrated to obtain the final product.

Weight of product : 1.3608 gr

m.p. : 219-220 °C

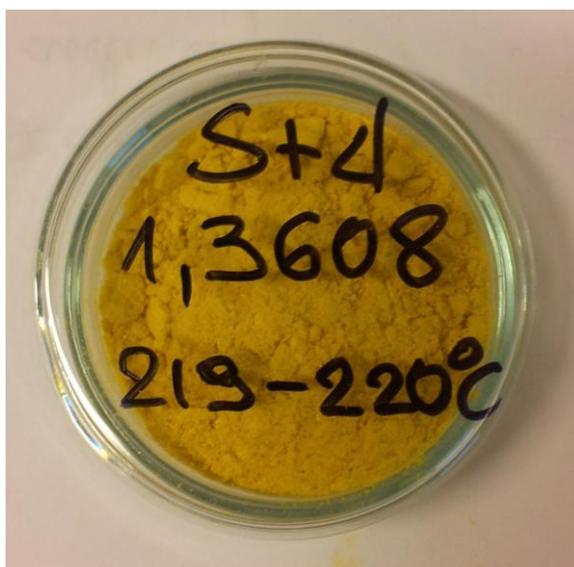
% yield= 45.39%

^1H NMR (300 MHz, DMSO) δ 13.03 (1H, bs, NH), 7.93 (1H, d overlapped, $J=8.8$ Hz, H6), 7.95-7.88 (2H, m, H3', H5'), 7.72-7.65 (2H, m, H2', H6'), 7.50 (1H, d, $J=2.2$ Hz, H3), 7.36 (1H, dd, $J=8.8$ Hz, $J=2.2$ Hz, H5)

^{13}C NMR (75 MHz, DMSO) δ 165.0, 163.9, 150.3, 143.0, 131.0, 126.3 (q, $J=3.5$ Hz), 125.6, 124.6 (q, $J=271.8$ Hz), 123.4 (q, $J=32.3$ Hz), 119.9, 113.4, 108.6

IR ν (CONH-) : 1670 cm^{-1}

According to IR and NMR results the product was triethylammoniu salt.



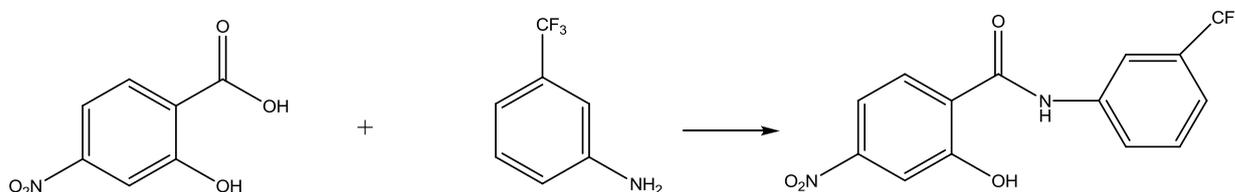
Experiment No. 5 (St 5)

Compound: 2-Hydroxy-4-nitro-*N*-(3-(trifluoromethyl)phenyl)benzamide

Chemical Formula: C₁₄H₉F₃N₂O₄

Molecular Weight: 326.23

Reaction scheme:



Contents:

- 1.8364 g of 2-hydroxy-4-nitrobenzoic acid
- 1.6230 g of 3-trifluoromethylaniline
- 50 ml of chlorobenzene
- 0.435 ml of PCl₃
- 1.2 ml of triethylamine

Procedure:

The mixture of the above contents was boiled in the microwave reactor at 120 °C and 400 watt for 20 minutes. After that, 20 ml of NaHCO₃ were added in order to form precipitation and filtered off.

The product of this filtration was recrystallized with ethanol three times to obtain the final one.

Weight of product : 1.1738 gr

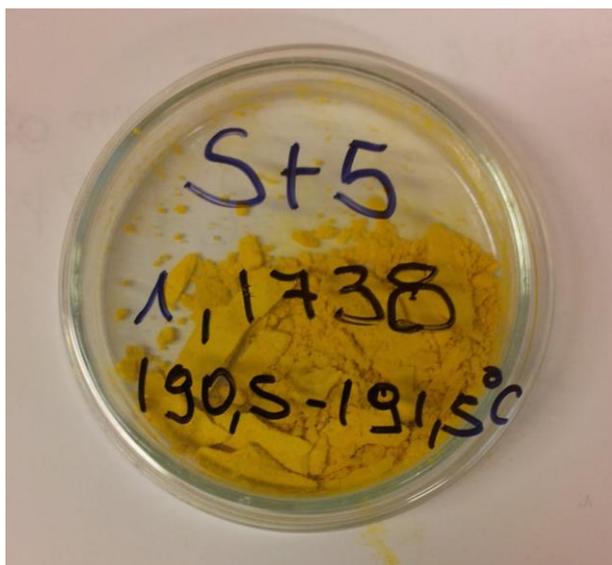
m.p. : 190.5-191.5 °C

% yield= 33.93%

^1H NMR (500 MHz, DMSO) δ 13.01 (1H, bs, NH), 8.28 (1H, bs, H2'), 7.93 (1H, d, $J=8.2$ Hz, H4'), 7.81 (1H, d, $J=8.8$ Hz, H6), 7.56 (1H, t, $J=8.2$ Hz, H5'), 7.50 (1H, d, $J=2.2$ Hz, H3), 7.43-7.39 (1H, m, H6'), 7.36 (1H, dd, $J=8.8$ Hz, $J=2.2$ Hz, H5)

^{13}C NMR (125 MHz, DMSO) δ 165.1, 163.9, 150.3, 140.2, 131.0, 130.2, 129.8 (q, $J=31.4$ Hz), 125.6, 124.4 (q, $J=271.8$ Hz), 123.5, 119.7 (q, $J=3.9$ Hz), 116.0 (q, $J=3.9$ Hz), 113.4, 108.6

IR v (CONH-) :1672 cm^{-1}



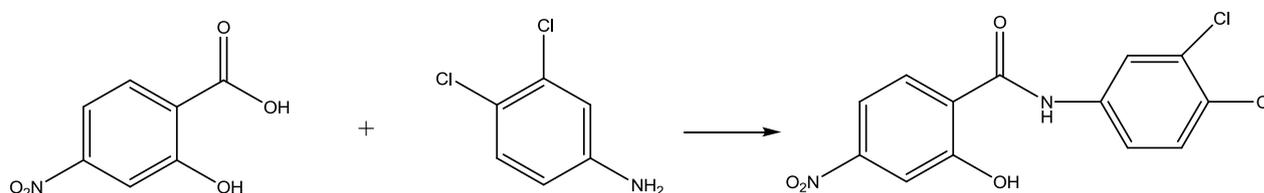
Experiment No. 6 (St 6)

Compound: *N*-(3,4-dichlorophenyl)-2-hydroxy-4-nitrobenzamide

Chemical Formula: C₁₄H₈Cl₂F₃NO₂

Molecular Weight: 350.12

Reaction scheme:



Contents:

- 1.8302 g of 2-hydroxy-4-nitrobenzoic acid
- 1.7740 g of 3,4-dichloroaniline
- 50 ml of chlorobenzene
- 0.435 ml of PCl₃
- 1.2 ml of triethylamine

Procedure:

The mixture of the above contents was boiled in the microwave reactor at 120 °C and 400 watt for 20 minutes. After that, 30 ml of NaHCO₃ were added in order to form precipitation and this precipitate was filtrated.

This precipitate, named as St 6(1), was mixed with enough amount of ethylacetate to get completely dissolved and filtrated.

This filtrate was evaporated till dryness and then recrystallized twice to obtain the final product.

Weight of product : 0.1160 gr

m.p. :222-224 °C

% yield= 3.22 %

^1H NMR (500 MHz, DMSO) δ 12.98 (1H, bs, NH), 8.16 (1H, bs, H2'), 7.90 (1H, d, $J=8.5$ Hz, H6), 7.58-7.51 (2H, m, H5', H6'), 7.48 (1H, d, $J=2.3$ Hz, H3), 7.35 (1H, dd, $J=8.5$ Hz, $J=2.3$ Hz, H5)

^{13}C NMR (125 MHz, DMSO) δ 164.9, 163.8, 150.3, 139.5, 131.3, 130.9, 130.9, 125.4, 124.8, 121.1, 120.0, 113.4, 108.6

IR v (CONH-) : 1668 cm^{-1}



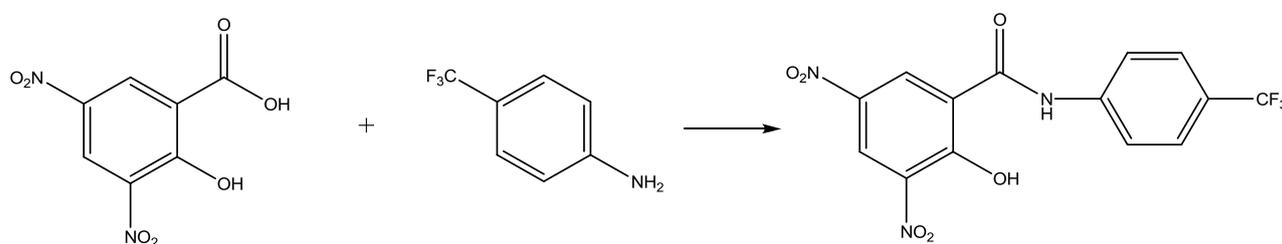
Experiment No. 7 (St 7)

Compound: 2-Hydroxy-3,5-dinitro-*N*-(4(trifluoromethyl)phenyl)benzamide

Chemical Formula: C₁₄H₈F₃N₃O₆

Molecular Weight: 371.23

Reaction scheme:



Contents:

- 2.2839 g of 2-hydroxy-3,5-dinitrobenzoic acid
- 1.6283 g of 3-trifluoromethyl-aniline
- 50 ml of chlorobenzene
- 0.435 ml of PCl₃
- 1.3 ml of triethylamine

Procedure:

The mixture of the above contents was boiled in the microwave reactor at 120 °C and 400 watt for 20 minutes. After that, 25 ml of NaHCO₃ were added in order to form precipitation and this precipitate was filtrated.

The filtrate was separated and the chlorobenzene layer (lower part) was kept. In this solution HCl was added and two layers appeared, which were separated again.

From this separation the lower phase was evaporated till dryness and ethylacetate was added. Then, it was separated, recrystallized and filtrated.

At the same time, the precipitation collected from the first filtration, named as St7(1), was recrystallized and filtrated twice.

Unfortunately, the amount of product from both experimental ways was not enough for IR and NMR tests.

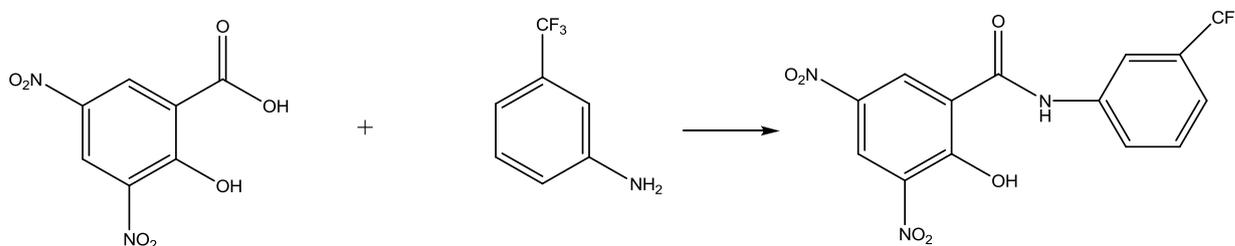
Experiment No. 8 (St 8)

Compound: 2-Hydroxy-2,3-dinitro-*N*-(3(trifluoromethyl)phenyl)benzamide`

Chemical Formula: C₁₄H₈F₃N₃O₆

Molecular Weight: 371.23

Reaction scheme:



Contents:

- 2.2836 g of 2-hydroxy-3,5-dinitrobenzoic acid
- 1.6120 g of 4-trifluoromethyl-aniline
- 50 ml of chlorobenzene
- 0.435 ml of PCl₃
- 1.2 ml of triethylamine

Procedure:

The mixture of the above contents was boiled in the microwave reactor at 120 °C and 400 watt for 20 minutes. After that, 30 ml of NaHCO₃ were added in order to form precipitation and this precipitate was filtrated.

The filtrate was separated and the chlorobenzene layer (lower part) was kept. In this solution HCl was added and two layers appeared, which were separated again.

From this separation the lower phase was evaporated till dryness and hexane was added. Then, it was evaporated again and ethylacetate was added. This solution was evaporated, recrystallized and filtrated.

At the same time, the precipitation collected from the first filtration, named as St8(1), was recrystallized and filtrated twice.

Unfortunately, the amount of product from both experimental ways was not enough for IR and NMR tests.

3. Conclusion

According to the results of the NMR measurement some products seem to be triethylammonium salts of salicylanilides. This fact was found in the end of my experimental part. On TLC prepared compounds showed only one running spot, thus we expected pure product. Triethylamine was used to catch up hydrogen chloride which is formed during the reaction mechanism.. Phenolic hydroxyl group is probably more acidic in our nitroderivatives than in other earlier prepared salicylanilides, nitro group has strong electron acceptor effect and thus increases acidity.

The expected results from the tests, which took place in Ostrava University, unfortunately are not available at the moment, due to the problems we confronted during the period of our experimental work.

Finally , these results, whose tests are still in progress, will revile the possibility of effectiveness of salicylanilides , against tuberculosis disease.

4. Bibliography - References

¹ Goldrick BA. Once dismissed, still rampant: tuberculosis, the second deadliest infectious disease worldwide. *Am J Nurs.* 2004; 104(9):68–70.

² Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. *Lancet.* 2003;362: 887–899

³ Jensen PA, Lambert LA, Iademarco MF, Ridzon R; Centers for Disease Control and Prevention. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care settings, 2005. *MMWR Recomm Rep.* 2005;54(RR-17):1–141

⁴ Dheda, K, Booth H, Huggett JF, et al. Lung remodeling in pulmonary tuberculosis. *J Infect Dis.* 2005;192:1201–1210

⁵ Wang JY, Hsueh PR, Wang SK, et al. Disseminated tuberculosis: a 10-year experience in a medical center. *Medicine (Baltimore).* 2011; 86(1):39–46

⁶ Toth A, Fackelmann J, Pigott W, Tolomeo O. Tuberculosis prevention and treatment. *Can Nurse.* 2004;100(9):27–30

⁷ Clinical Article, Tuberculosis: Pathophysiology, Clinical Features, and Diagnosis, April 2009

⁸ Endohang – Ndong J., (2012), Antimycobacterial drugs currently in Phase II clinical trials and preclinical phase for tuberculosis treatment, Review, Ken State University

⁹ British National Formulary; 64th Edition (Sep 2012) British Medical Association and Royal Pharmaceutical Society of Great Britain, London

¹⁰ Zimmerman HJ. Antituberculosis agents. In, Zimmerman HJ. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. 2nd ed. Philadelphia: Lippincott, 1999, pp. 611-21.

¹¹ Molecular Medicine Reports 1: 3-13, 2008 3Prevention and modulation of aminoglycoside ototoxicity ,Gianpaolo Perletti, Anne Vral, Maria Cristina Patrosso, Emanuella Marras, Isabella Ceriani, Petra Willems, Mauro Fasano and Vittoria Margil, Department of Structural and Functional Biology, Laboratory of Toxicology and Pharmacology, and Center of Neuroscience,(MF), Università degli Studi dell'Insubria, Busto Arsizio, Varese, Italy; Department of Anatomy, Embryology, Histology and Medical Physics, Ghent University, Ghent, Belgium; Medical Genetics Laboratory, Medicine Laboratory, Department, Niguarda Ca'Granda Hospital, Hospital Istituti Clinici di Perfezionamento, Milan, Italy, (November 2007)

¹² Payam Nahid, Madhukar Pai, and Philip C. Hopewell "Advances in the Diagnosis and Treatment of Tuberculosis", Proceedings of the American Thoracic Society, Vol. 3, No. 1 (2006), pp. 103-110.

¹³Price, C. T., I. R. Lee, and J. E. Gustafson, (2000). The effects of salicylate on bacteria. Int. J. Biochem. Cell. Biol. 32:1029-1043

¹⁴ Fukai Ryosuke, Zheng Xiaoxia, Motoshima Kazunori, Tai Akihiro, Yazama Futoshi, Kakuta Hiroki, Design and Synthesis of Novel Cyclooxygenase-1 Inhibitors as Analgesics: 5-Amino-2-ethoxy-N-(substituted-phenyl)benzamides. ChemMedChem, 2011 , vol. 6, # 3 p. 550 - 560