CHARLES UNIVERSITY

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

DPT. OF PHARMACEUTICAL CHEMISTRY AND PHARMACEUTICAL ANALYSIS

SYNTHESIS AND ANTIINFECTIVE EVALUATION OF SUBSTITUTED *N*-(PYRAZIN-2-YL)BENZENESULFONAMIDES

Diploma Thesis

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Hradec Králové, 2018

Acknowledgement

I would like to express my gratitude to Ms. Ghada Bouz for taking her time to teach me, encourage me to do more things than what I thought I could handle and never doubting my capabilities.

Also would like to thank Prof. Martin Doležal for his support and good wishes. To Dr. Jan Zitko for his constant flow of ideas and supervision.

And last but not least to my family and boyfriend for the encouragement to do this Erasmus stay that has rewarded me with so much more than I could imagine.

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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. The thesis was not misused for obtaining the same or different academic degree."

Cristina Paredes de la Red

Hradec Králové, 15th of May of 2018

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1. LIST OF ABBREVIATIONS

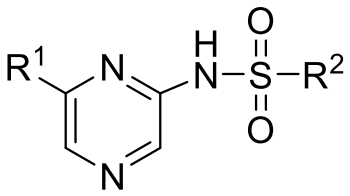
CPX	Ciprofloxacin
DMSO	Dimethyl sulfoxide
EMB	Ethambutol
EtOAc	Ethyl Acetate
FAS I	Fatty Acid Synthase I
FDA	Food and drug administration
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HIV	Human immunodeficiency virus
IC_{50}	Half maximal inhibitory concentration
INH	Isoniazid
IR	Infrared
MDR-TB	Multi-drug resistant tuberculosis
MIC	Minimum inhibition concentration
Mtb	*Mycobacterium tuberculosis*
mtQAPRTase	Quinolinic Acid Phosphoribosyltransferase from *Mycobacterium tuberculosis*
NA	Not available
NMR	Nuclear Magnetic Resonance

OADC	Oleic acid, albumin, dextrose, catalase
OIDD	Open Innovation Drug Discovery
PABA	*Para*-aminobenzoic acid
RFM	Rifampicin
Ro3	Rule of three
Ro5	Rule of five
RpsA	Ribosomal protein S1
PanD	Aspartate Decarboxylase
POA	Pyrazinoic acid
PZA	Pyrazinamide
QA	Quinolinic Acid
RIF	Rifampicin
SGK1	serum and glucocorticoid regulated kinase 1
TB	Tuberculosis
TLC	Thin layer chromatography
tmRNA	Transfer-messenger RNA
TPSA	Topological polar surface area
UV	Ultra violet
XDR-TB	Extensively drug-resistant tuberculosis

2. AIM OF WORK

According to my five-month Erasmus stay plan, my work aimed at synthesizing and purifying eight different *N*-(pyrazine-2-yl)benzenesulfonamides (general chemical structure is shown below) as part of a bigger series composed of 23 different compounds in total. This series is based on the hybridization approach combing pyrazine core with sulfonamide moiety.

Beside chemical synthesis and purifications, the work also focused on biological evaluations of the prepared compounds for their antimycobacterial activities against five different mycobacterial strains, antibacterial activities against eight bacterial strains, and antifungal activities against eight fungal strains, and subsequent results interpretation.



R^1 : H, Cl; R^2 : Aromatic substituents

3. BACKGROUND

Tuberculosis (TB) is an old infection caused by the bacillus *Mycobacterium tuberculosis*. This disease is highly contagious through infected air droplets when coughing or even speaking. Although TB is often an infection of the respiratory track, the infection could occur elsewhere, like in the meninges, causing what is known as extrapulmonary TB. It is estimated that one third of the world's population is infected with TB, and only 10% will develop the active form of the infection. The increase in number of patients with immune suppressant diseases, for example HIV co-infection, and other risk factors, including under-nutrition, diabetes, smoking and alcohol, make people more vulnerable to acquire TB[1]. Despite the availability of a well stablished treatment regimen, the emerging drug resistant strains and multi-drug resistant (MDR-TB) make this curable infection more hard to treat. It is believed that the increase in resistant TB may be attributed to the large periods of treatment that results in an irregular intake of drugs[2].

Yet it is important to state that 95% of deaths caused by TB occur in developing countries due to inaccessibility to health care or nonadherence to the long treatment[1].

The previously mentioned facts prompt us to find new treatments *Mycobacterium tuberculosis* (*Mtb)* are susceptible, besides having good safety profile and reduced toxicity. One of the leading new findings involve the pyridazine system and it has been reported to have anti-TB activity[3].

3.1 Tuberculosis Treatment Throughout History

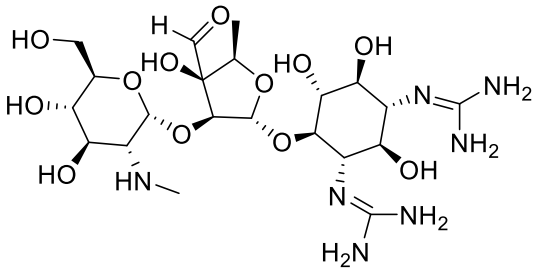
The history of TB treatment begins with the discovery of streptomycin (**Figure 1**) 70 years ago as the first antibiotic active against TB discovered. Yet the fast resistance development against Streptomycin limited its clinical benefit. In the 50s new compounds were registered with antiTB activity with different mechanisms of action such as: isoniazid, pyrazinamide, kanamycin, *para*-amino salicylic acid and cycloserine (**Figure 1**). As an attempt to prevent resistance development, such drugs were administered in combination for a total duration of 18 months. Then with the introduction of rifampicin in the 60s, the treatment was shortened to 9 months[4]. Pyrazinamide further shortened the duration of therapy to 6 months.

Therefore, the up to date treatment regimen for drug-susceptible pulmonary TB is 6 months with the four first line agents as follow; initial phase entails isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) (**Figure 1**) for 2 months followed by a continuation phase that consists of isoniazid and rifampicin for 4 months[5].

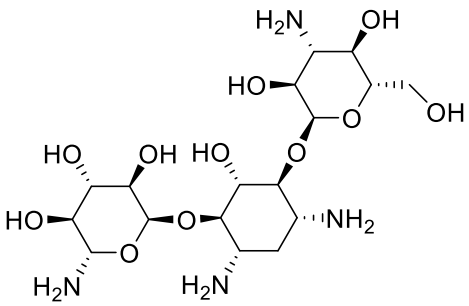
Before initiating the treatment, drug resistance against INH and RIF should be ruled out[6]. If the patient is co-infected with HIV[7], it is recommended to start antiretroviral treatment as soon as possible after the initiation of the normal drug-susceptible TB treatment.

In populations with high isoniazid resistance, patients receive the same initial phase regimen, yet in the continuation phase, ethambutol is not removed and continued till the end of treatment period of 6 months in total[5].

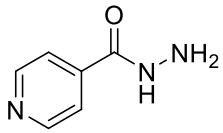
For MDR-TB, the decentralized treatment is recommended over the centralized one. This means that every patient shall be treated individually depending on the drug-resistant TB that she/he is infected with. It is also encouraged to educate the population about the nature of TB and about the problems that can follow if the treatment is not followed as recommended[5]. Extensively drug-resistant TB (XDR-TB) is when the infection is resistant to isoniazid, rifampicin, fluoroquinolones and one of the injectable second-line anti-TB drugs (amikacin, kanamycin or capreomycine) (**Figure 1**).



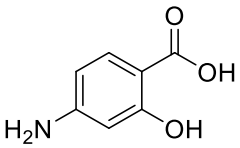
Streptomycin



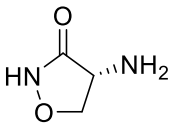
Kanamycin



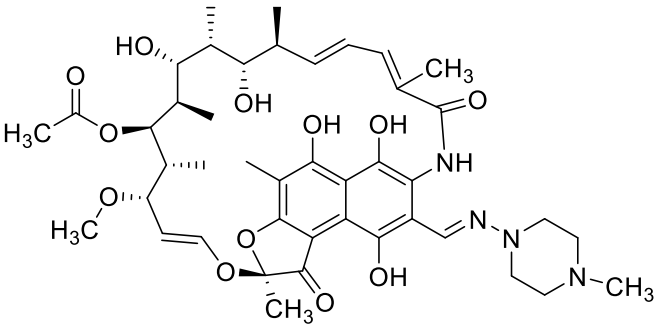
Isoniazid



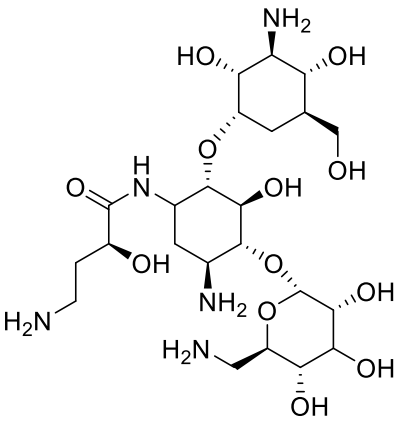
Para-amino salicylic acid



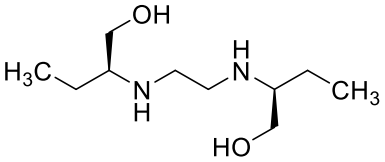
Cycloserine



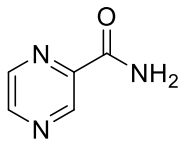
Rifampicin



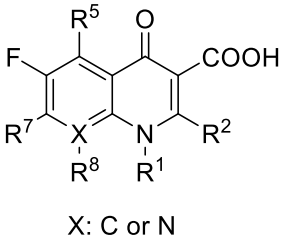
Amikacin



Ethambutol

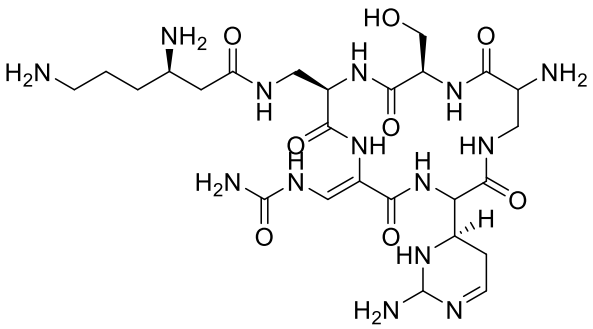


Pyrazinamide



X: C or N

Fluoroquinolones



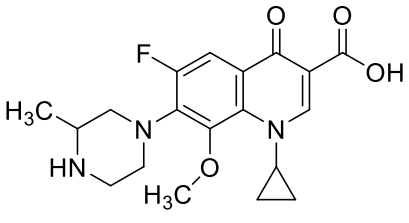
Capreomycin

Figure 1: Different agents with activity against TB.

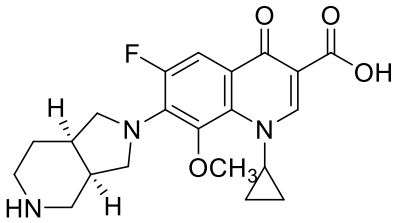
3.2 New Approaches for Resistant Tuberculosis

The increasing number of cases of drug resistant TB, and the fact that the treatment regimen is too lengthy highlighted the need for better treatments and more effective approaches.

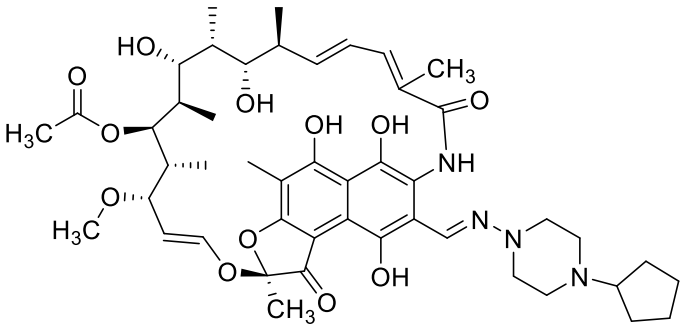
Novel and repurposed drugs are currently in clinical trials for both drug-susceptible TB and drug-resistant TB. Ongoing clinical trials are also exploring different combinations of anti-TB drugs to shorten the period of treatment to 4 months in total by replacing ethambutol or isoniazid for the repurposed drugs gatifloxacin or moxifloxacin. Another approach is using twice a week dosing regimen of rifapentine with moxifloxacin during the continuation phase to shorten the treatment period[5] (**Figure 2**).



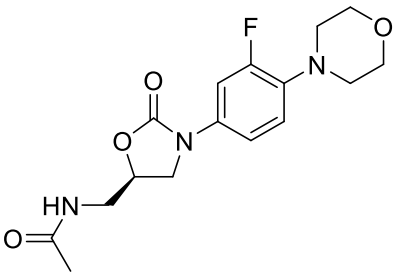
Gatifloxacin



Moxifloxacin



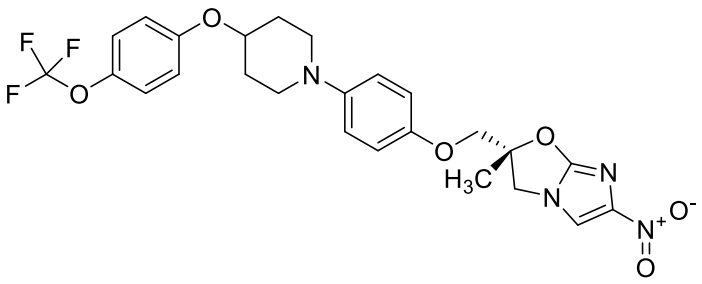
Rifapentine



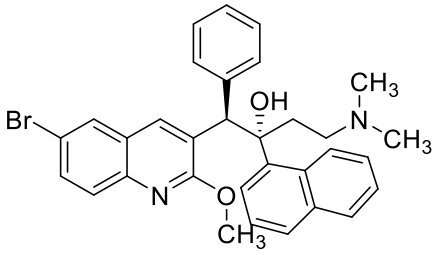
Linezolid

Figure 2: Repurposed drugs with activity against TB.

New FDA approved drugs include delamanid which is a nitroimidazole derivative that causes a lethal release of nitrogen species inside the mycobacterial cell. Furthermore, bedaquiline is a new anti-TB that targets ATP synthesis. Both agents have been tested on MDR-TB patients and now are used in patients where other alternatives fail to work (**Figure 3**).



Delamanid



Bedaquiline

Figure 3: New FDA approved drugs to treat TB.

Nitroimidazole derivatives and the repurposed oxazolidinone, linezolid (**Figure 2**), are two other examples of compounds with activity against XDR-TB[4].

3.3 Pyrazine

Pyrazine, also known as 1,4-diazine, is a 6 membered heterocyclic aromatic system with two nitrogens in *para* position. Pyrazine ring is 6π-electro-deficient (**Figure 4**)[8].

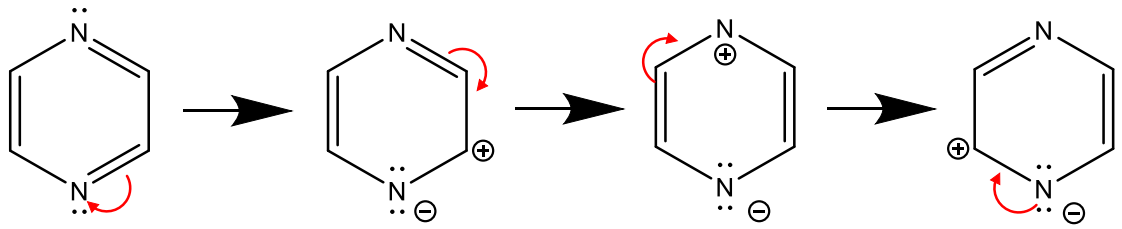


Figure 4: Pyrazine resonance.

The pK_a of pyrazine is 0.65 lower than any of the other diazines, and even lower than pyridine making it a very weak base. This low basicity is attributed to the combination of inductive and mesomeric withdrawal effects that is made by the second nitrogen. Its boiling point is high (118°C) but is still lower than the rest of the diazines[8].

Pyrazine derivatives are quite abundant and can be found in foods as flavouring substances, natural occurrence in beef, chicken, cocoa, coffee, green tea, fruit juice, potato, pork, whisky, sherry, nuts, peanut, roasted sesame seed, malt and wild rice[9].

Pyrazine is de-novo produced by living organisms with physiological and biological importance such as alerting pheromones, site markers, repellents, etc. There are recent studies of diazine derivatives proving their wide spectrum of pharmacological properties like antibacterial, antifungal and antiviral activity.

The antitubercular PZA is a synthetic pyrazine analogue of nicotinamide introduced in the 50s as part of the TB treatment in the initial phase of treatment. The PZA is a vital component of TB regiment due to its sterilizing effect against semi-dormant bacilli that are not killed by the other anti-TB drugs[10]. Pyrazinamide exerts a tight structure-activity relationship with the pyrazine ring being an important pharamcophore[2].

Several new mechanisms of action by which PZA acts have been recently identified. PZA is a prodrug that requires activation to its active form, pyrazinoic acid (POA), in acidic pH by the *M. tuberculosis* pyrazinamidase/nicotinamidase enzyme encoded by the *pncA* gene. A newly discovered mechanism by which PZA functions is the inhibition of the ribosomal protein S1 (RpsA). This protein plays a vital role in translation as it binds directly to the upstream sequence of mRNA from one side and the ribosome from the other side. It is also involved in trans-translation, which is a process used by the bacteria to survive and perform translation

despite the presence of defect mRNA or stalled ribosomes [11]. Yet, a recent study opposes this theory claiming that such inhibition does not take place[12]. Other proposed mechanisms of action include the inhibition of quinolinic acid phosphoribosyltransferase (QAPRTase) that is essential for the synthesis of nicotinamide adenine dinucleotide (NAD) and the inhibition of aspartate decarboxylase that is responsible for the synthesis of vitamin B5 and subsequently coenzyme A (CoA)[13]. The discovery of gene mutations contributing to resistance against PZA, lead to the identification of another mechanism of action, which is the inhibition of aspartate decarboxylase (PanD) [14, 15]. Lastly POA also inhibits the Fatty acid synthase I (FAS I) involved in the synthesis of short mycolic acids in the membrane of *M. tuberculosis*[16].

The newly discovered mechanisms have driven efforts worldwide to manipulate the chemical structure of the very interesting pyrazinamide in order to improve its biological activity[17].

3.4 Sulfonamides

Sulfonamides were the very first antibiotics used to prevent and treat bacterial infections. The general chemical structure consists of a benzene core, an amino substituent (-NH_2) in position 4 and a sulfonamide moiety (SO_2NH-) in position 1. The -NH_2 in position 4 can be substituted by other radicals giving raise to different sulfonamides with different pharmacological activities. The (SO_2NH-) needs to be attached to the benzene ring (**Figure 5**)[18].

Sulfonamides exert their antibacterial activity by competitive inhibition since they are structural analogous of *para*-aminobenzoic acid (PABA), the precursor of folic acid. By acting as a false precursor, sulfonamides inhibit the bacterial de-novo synthesis of folic acid and subsequently DNA and RNA synthesis. Based on their mechanism of action, sulfonamides are bacteriostatic rather than bactericidal[19].

Common anti-infectives containing sulfonamide in their chemical structures include the topical Ag-sulfadiazine and sulfamethoxazole, which is the drug of choice for some infections such as toxoplasmosis and pneumocystosis in HIV patients (**Figure 5**). Besides antiinfective properties, sulfonamide containing compounds exert anti-tumor, anti-inflammatory, anti-convulsive, diuretic, and hypoglycemic effects[20]. The chemical strructure of these sulfonamides differ from the general chemical structure of the anti-infective sulfonamides shown below in **Figure 5**.

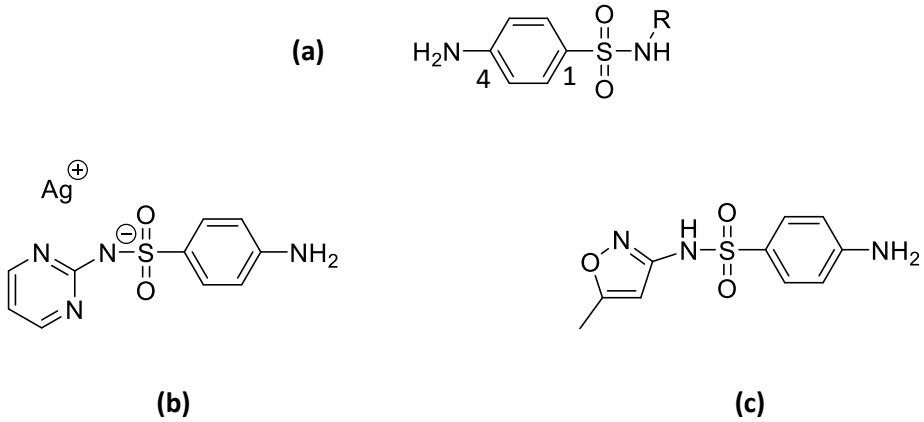
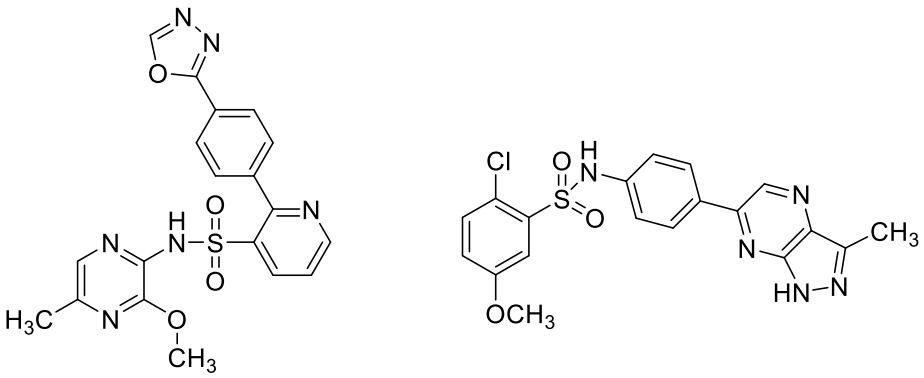


Figure 5: (a) The general structure of anti-infective sulfonamides (b) The chemical structure of Ag-sulfadiazine (c) The chemical structure of sulfamethoxazole.

3.5 Pyrazine Containing Sulfonamides

Several pyrazine containg sulfonamides are already established with wide range of pharmacological applications. Examples include zibotentan (**Figure 6**), which is an endothelin receptor antagonist with antitumor activity for prostate cancer. A number of substituted pyrazinylmethyl sulfonamides exerted fungicidal activity[21]. Besides, some halogenated *N*-pyrazinyl-phenyl sulfonamides were found to bind to one or more receptors of the CCP chemokine subfamily, and thus have medical application in treating chemokine mediated diseases, such as asthma[22].

A series of *N*-[4-(1*H*-Pyrazolo[3,4-b]pyrazin-6-yl)-phenyl]sulfonamides was prepared as active and selective inhibitors of the serum and glucocorticoid regulated kinase 1 (SGK1). SGK1 disregulation is connected with many pathological conditions, including cancer, diabetes, hypertension, and neurodegeneration. The chemical structure of the leading compound of this series with best biological and safety profile is demonstrated in **figure 6**[23].



(a) **(b)**

Figure 6: (a) The chemical structure of zibotentan (b) The chemical structure of the lead compund from a series of *N*-[4-(1*H*-Pyrazolo[3,4-b]pyrazin-6-yl)-phenyl]sulfonamides.

4. EXPERIMENTAL PART

4.1 Instrumentation

Most of the chemical reactions were carried in normal laboratory glass equipment and at room temperature.

The progress of the reaction was checked by Thin Layer Chromatography (TLC) (Alugram® Sil G/UV254, Machery-Nagel, Postfach, Germany) with UV detection using wavelength 254 nm.

For selected compounds, microwave assisted reactions were performed in a CEM Discover microwave reactor with a focused field (CEM Corporation, Matthews, NC, USA) connected to an Explorer 24 autosampler (CEM Corporation).

All obtained products were purified by preparative flash chromatograph CombiFlash® Rf (Teledyne Isco Inc., Lincoln, NE, USA). The type of elution was gradient, using the mixture of hexane and ethyl acetate (EtOAC) as mobile phase. Silica (0.040–0.063 nm, Merck, Darmstadt, Germany) was used as the stationary phase.

NMR spectra of prepared compounds were recorded on Varian VNMR S500 (499.87 MHz for ^{1}H and 125.71 MHz for ^{13}C) spectrometer (Varian Corporation, Palo Alto, CA, USA).

Infrared spectra was recorded with spectrometer FT-IR Nicolet 6700 (Thermo Scientific, Waltham, MA, USA) using attenuated total reflectance (ATR) methodology.

Elemental analysis will be carried out using a vario Micro Cube Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

UHPLC system Acquity UPLC I-class (Waters, Millford, MA, USA) coupled to high resolution mass spectrometer (HRMS) Synapt G2Si (Waters, Manchester, UK) based on Q-TOF will be used for HRMS spectra measurement.

Melting points were assessed by SMP3 Stuart Scientific (Bibby Sterling Ltd., Staffordshire, UK) in open capillary.

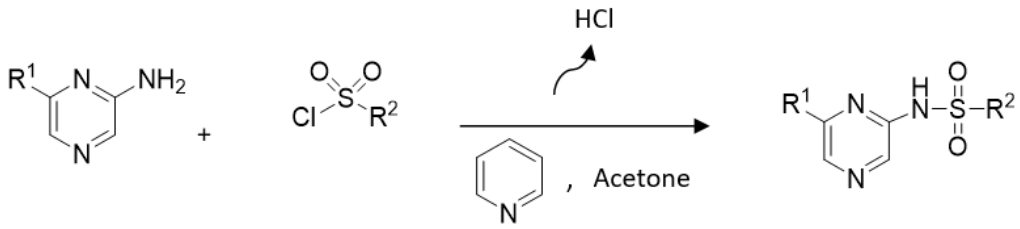
Lipophilicity parameter log *P* were calculated by software CS ChemBioDraw Professional 17.0 (CambridgeSoft, Cambridge, MA, USA).

All chemical structures in this diploma were generated using the software CS ChemBioDraw Professional 17.0, and none was copied from the internet. An exception includes the structures in descriptors section, which were automatically generated by the Open Innovation Drug Discovery (OIDD) *in silico* evaluation from their corresponding smiles.

4.2 N-(pyrazin-2-yl)benzenesulfonamides

4.2.1 Chemistry

Based on the synergism methodology between pyrazinamide, represented by the pyrazine core, and the sulfonamide moiety, a series of 22 different *N*-(pyrazin-2-yl)benzenesulfonamides was designed and synthesized. The chemical synthesis entails a simple one step reaction in acetone and pyridine between different sulfonyl chlorides and one time with aminopyrazine, while the other with 6-chloroaminopyrazine (**Scheme 1**). The reason of duplicating each reaction is to evaluate the effect of the chlorine atom on biological activity. As it is known, the introduction of a chlorine atom to any molecule will increase its lipophilicity. For antimycobacterial activity, lipophilicity of the intended compounds is an important factor since Mycobacteria have thick, lipid rich mycolic cell walls. Besides, chloropyrazine derivatives, such as 5-Cl-PZA, esters of 5-Cl-POA, anilides of 5- or 6-Cl-POA were previously shown to possess *in vitro* antimycobacterial activity[24-27].



Scheme 1: General reaction. R^1: H, Cl; R^2: Aromatic substituents.

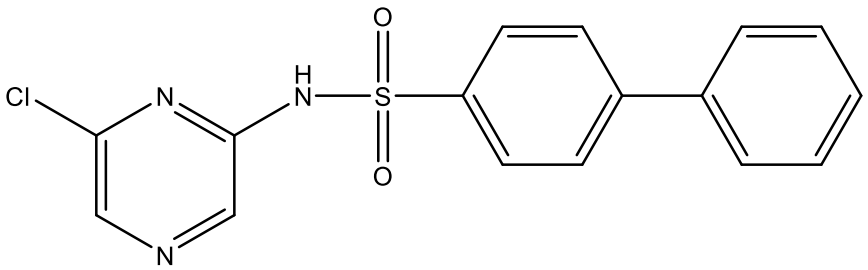
4.2.2 General Procedure

In 50 mL beaker, aminopyrazine (3 mmol, 285 mg) or 6-chloroaminopyrazine (3 mmol, 385 mg) were dissolved in 1 mL pyridine with stirring at room temperature. Pyridine was used to neutralize the hydrochloric molecules generated upon reacting the sulfonylchloride reagent with the corresponding aminopyrazine or 6-chloroaminopyrazine. On the other hand, 3 mmol of benzylsulfonylchloride reagent were dissolved in approximately 2 mL of acetone in a 50 mL pear shaped beaker with stirring at room temperature. Then the dissolved content in pyridine of the 50 mL beaker was added drop wise to the dissolved benzylsulfonylchloride with stirring at room temperature. The system was then sealed with appropriate stopper and let react overnight under same conditions. The next day, the completion of reaction was checked by

TLC in 3:1 EtOAc/hexane system. The reaction mixture was transferred to a 100 mL beaker and washed with distilled water to minimize any losses. Then the content was acidified with 10% HCl drop-wise until a solid precipitate is formed which represents the non-ionized form of the product. The acidification step also aimed at neutralizing the added pyridine and ionizing any unreacted aminopyrazine or 6-chloroaminopyrazine to form a salt that dissolved in the aqueous layer in the subsequent extraction. EtOAc was added as little as needed to dissolve the formed solid precipitate. Distilled water was added then in needed quantities to equalize the volumes of the aqueous layer with the EtOAc organic layer. The two phases were mixed vigorously at room temperature and then transferred to a 500 mL separating funnel. The two layers were then allowed to settle and separated into two 250 mL beakers. The aqueous layer was washed again with EtOAc. The organic layer was washed one more time with distilled water. The recovered organic layers from all extractions were added all together and then extracted one last time with distilled water and then with brine. The final organic layer was then transferred to a 500 mL (or less based on the estimated overall volume) and stirred with magnesium sulfate (4 mmol, 500 mg) as a desiccate for 10 minutes at room temperature. Finally, the solution was filtrated with cotton and adsorbed to silica gel to perform flash chromatography using gradient elution 0% to 100% EtOAc in hexane.

As stated earlier in the “aim” section, I have contributed in the synthesis, purification and biological evaluation of eight different *N*-(pyrazin-2-yl)benzenesulfonamides out of a bigger series. In the following section I demonstrate the profiles of only the eight compounds I have prepared.

4.2.2.1 *N*-(6-chloropyrazin-2-yl)-[1,1'-biphenyl]-4-sulfonamide



Code: 4b

Chemical Formula: $C_6H_{12}ClN_3O_2S$

Molecular weight: 345,80 g/mol

***Yield:** 10,7 %

Appearance: Light yellow powder

m.p.: 242,2 – 244,3 ºC

R_f (EtOAc/Hexane 3:1): 0,70

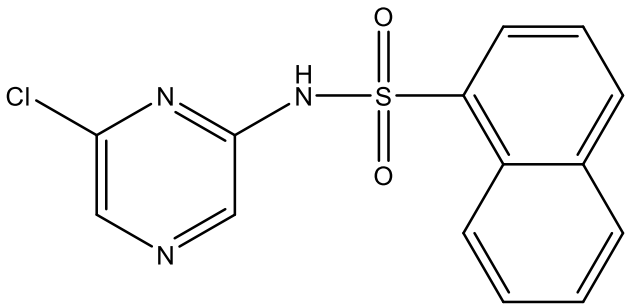
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.01 (s, 1H, sulfonamide), 8.34 (d, *J* = 6.9 Hz, 2H, pyrazine), 8.08 – 8.02 (m, 2H, arom.), 7.94 – 7.88 (m, 2H, arom.), 7.75 – 7.69 (m, 2H, arom.), 7.52 – 7.46 (m, 2H, arom.), 7.46 – 7.39 (m, 1H, arom.).

^{13}C NMR (125 MHz, DMSO-d_6) δ 163.6, 151.4, 151.0, 143.9, 138.8, 136.3, 134.8, 131.4, 130.9, 124.7, 120.3, 119.3, 66.7, 21.7, 10.5.

Elemental analysis: C, 55.60%; H, 3.50%; N, 11.93%; S, 9.54%. **Calculated for $C_{14}H_{10}ClN_3O_2S$ (MW 345,03):** C, 55.57%; H, 3.50%; N, 12.15%; S, 9.27%.

*Yields are expressed as percentages of theoretical yields and refer to the isolated products (chromatographically pure) after all purification steps.

4.2.2.2 *N*-(6-chloropyrazin-2-yl)naphthalene-1-sulfonamide



Code: 5b

Chemical formula: $C_{14}H_{10}ClN_3O_2S$

Molecular weight: 319,76 g/mol

***Yield:** 13,0 %

Appearance: Light yellow powder

m.p.: 185,9 – 187,0 ºC

Rf (EtOAc /Hexane 3:1): 0,69

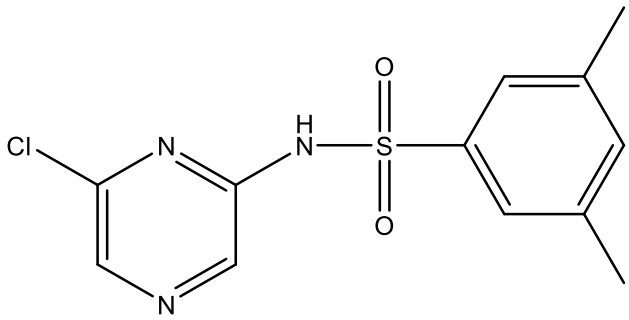
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.05 (s, 1H, sulfonamide), 8.35 (s, 2H, pyrazine), 7.93 (dd, *J* = 8.8, 2.0 Hz, 3H, arom.), 7.76 – 7.64 (m, 4H, arom.).

^{13}C NMR (125 MHz, DMSO-d_6) δ 147.5, 146.0, 145.6, 137.4, 136.3, 134.7, 132.5, 131.6, 130.6, 129.6, 129.6, 128.7, 128.0, 128.0.

Elemental analysis found: C, 52.89%; H, 3.10%; N, 13.07%; S, 10.04%. **Calculated for $C_{14}H_{10}ClN_3O_2S$ (MW 319.02):** C, 52.59%; H, 3.15%; N, 13.14%; S, 10.03%.

*Yields are expressed as percentages of theoretical yields and refer to the isolated products (chromatographically pure) after all purification steps.

4.2.2.3 *N*-(6-chloropyrazin-2-yl)-3,5-dimethylbenzenesulfonamide



Code: 6b

Chemical formula: $C_{12}H_{12}ClN_3O_2S$

Molecular weight: 297,76 g/mol

***Yield:** 15,3 %

Appearance: Waxy orange flakes

m.p.: 163,1 – 164,8 ºC

Rf (EtOAc /Hexane 3:1): 0,80

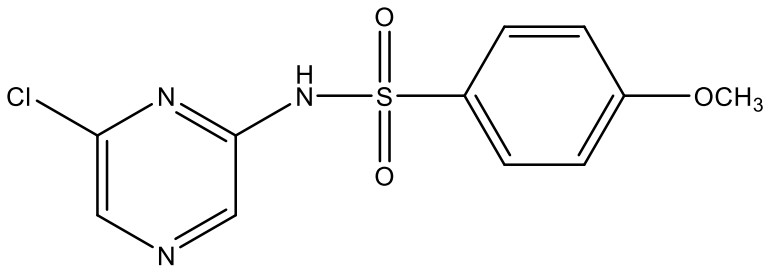
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.82 (s, 1H, sulfonamide), 8.35 (d, *J* = 0.7 Hz, 2H, pyrazine), 8.58 – 8.62 (m, 2H, arom.), 7.35 (d, *J* = 1.9 Hz, 1H, arom.), 2.38 (s, 6H, -CH_3).

^{13}C NMR (125 MHz, DMSO-d_6) δ147.5, 145.6, 139.2, 138.9, 137.2, 135.1, 132.8, 125.2, 20.9.

Elemental analysis found: C, 48.35%; H, 4.49%; N, 14.15%; S, 10.05%. **Calculated for $C_{12}H_{12}ClN_3O_2S$ (MW 297,03):** C, 48.41%; H, 4.06%; N, 14.11%; S, 10.77%.

*Yields are expressed as percentages of theoretical yields and refer to the isolated products (chromatographically pure) after all purification steps.

4.2.2.4 *N*-(6-chloopyrazin-2-yl)-4-methoxybenzenesulfonamide



Code: 7b

Chemical formula: $C_{11}H_{10}ClN_3O_3S$

Molecular weight: 299,73 g/mol

***Yield:** 53,9 %

Appearance: Yellow powder

m.p.: 185,7 – 187,8 ºC

Rf (EtOAc /Hexane 3:1): 0,58

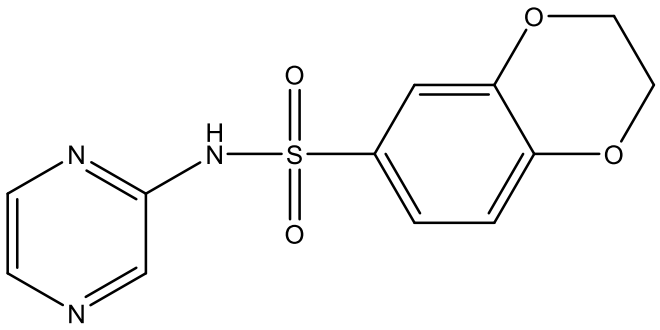
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.91 (s, 1H, sulfonamide), 8.31 (d, *J* = 1.4 Hz, 2H, pyrazine), 7.95 – 7.91 (m, 2H, arom.), 7.18 – 7.13 (m, 2H, arom.), 3.81 – 3.79 (m, 3H, -OCH_3).

^{1}C NMR (125 MHz, DMSO-d_6) δ 163.2, 147.6, 145.7, 137.1, 132.4, 130.9, 123.0, 114.6, 55.9.

Elemental analysis found: C, 44.48%; H, 3.30%; N, 14.14%; S, 10.56%. **Calculated for $C_{11}H_{10}ClN_3O_3S$ (MW 299.01):** C, 44.08%; H, 3.36%; N, 14.02%; S, 10.70%.

*Yields are expressed as percentages of theoretical yields and refer to the isolated products (chromatographically pure) after all purification steps.

4.2.2.5 *N*-(pyrazine-2-yl)-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide



Code: 8a

Chemical formula: $C_{12}H_{11}N_3O_4S$

Molecular weight: 293,30 g/mol

***Yield:** 7,8 %

Appearance: White fluffy flakes

m.p.: 213,1 – 215,0 ºC

Rf (EtOAc /Hexane 3:1): 0,50

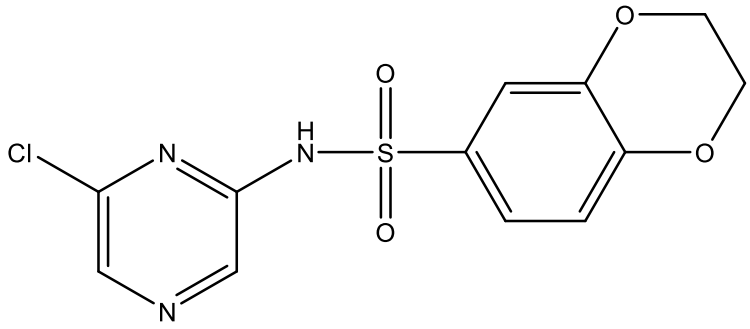
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.41 (s, 1H, sulfonamide), 8.35 (d, *J* = 1.4 Hz, 1H, pyrazine), 8.26 – 8.21 (m, 2H, pyrazine), 7.44 – 7.37 (m, 2H, arom.), 7.02 (d, *J* = 8.4 Hz, 1H, arom.), 4.33 – 4.25 (m, 4H, -CH_2-).

^{1}C NMR (125 MHz, DMSO-d_6) δ 156.2, 153.9, 147.0, 144.5, 137.4, 136.1, 131.9, 117.3, 115.3, 111.9, 64.2, 64.1.

Elemental analysis found: C, 49.73%; H, 4.23%; N, 13.85%; S, 10.19%. **Calculated for $C_{12}H_{10}N_3O_4S$ (MW 293.05):** C, 49.14%; H, 3.78%; N, 14.33%; S, 10.93%.

*Yields are expressed as percentages of theoretical yields and refer to the isolated products (chromatographically pure) after all purification steps.

4.2.2.6 *N*-(6-chloropyrazin-2-yl)-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide



Code: 8b

Chemical formula: $C_{12}H_{10}ClN_3O_4S$

Molecular weight: 327,74 g/mol

***Yield:** 7,8 %

Appearance: White-yellow flakes

m.p.: 213,5 – 215,6 ºC

Rf (EtOAc /Hexane 3:1): 0,73

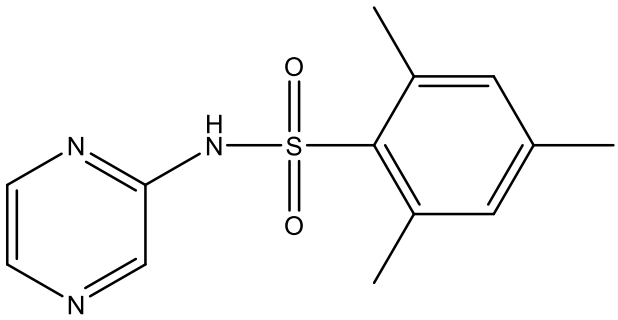
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.81 (s, 1H, sulfonamide), 8.34 (s, 2H, pyrazine), 8.28 (s, 2H, arom.), 7.47 – 7.41 (m, 1H, arom.), 4.42 – 4.26 (m, 4H, -CH_2-).

^{1}C NMR (125 MHz, DMSO-d_6) 156.8, 153.9, 147.0, 144.9, 135.1, 134.9, 131.9, 117.3, 115.3, 111.9, 64.2, 64.1.

Elemental analysis found: C, 44.93%; H, 3.03%; N, 12.38%; S, 9.76%. **Calculated for $C_{12}H_{10}ClN_3O_4S$ (MW 327.01):** C, 43.98%; H, 3.08%; N, 12.82%; S, 9.78%.

*Yields are expressed as percentages of theoretical yields and refer to the isolated products (chromatographically pure) after all purification steps.

4.2.2.7 2,4,6-trimethyl-*N*-(pyrazine-2-yl)benzenesulfonamide



Code: 9a

Chemical formula: $C_{13}H_{15}N_3O_2S$

Molecular weight: 277,34 g/mol

***Yield:** 14,0 %

Appearance: White cotton like powder

m.p.: 232,8 – 234,5 ºC

Rf (EtOAc /Hexane 3:1): 0,71

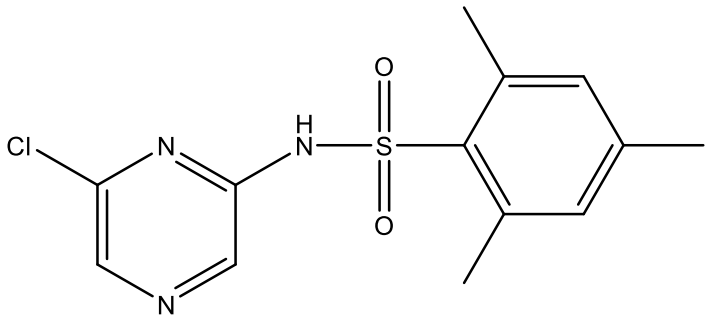
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.52 (s, 1H, sulfonamide), 8.22 (d, *J* = 1.5 Hz, 1H, pyrazine), 8.15 – 8.08 (m, 2H, pyrazine), 7.01 (s, 2H, arom.), 2.64 (s, 6H, -CH_3), 2.22 (s, 3H, -CH_3).

^{1}C NMR (125 MHz, DMSO-d_6) δ 148.9, 142.5, 142.0, 139.4, 138.1, 134.2, 134.1, 131.8, 22.4, 20.6.

Elemental analysis found: C, 56.22%; H, 5.42%; N, 14.88%; S, 11.92%. **Calculated for $C_{10}H_{16}N_4O$ (MW 277.09):** C, 56.30%; H, 5.45%; N, 15.15%; S, 11.56%.

*Yields are expressed as percentages of theoretical yields and refer to the isolated products (chromatographically pure) after all purification steps.

4.2.2.8 *N*-(6-chloropyrazin-2-yl)-2,4,6-trimethylbenzenesulfonamide



Code: 9b

Chemical formula: $C_{13}H_{14}ClN_3O_2S$

Molecular weight: 311,78 g/mol

***Yield:** 8,0 %

Appearance: White-yellow flakes

m.p: 241,5 – 242,6 ºC

Rf (EtOAc /Hexane 3:1): 0,68

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.95 (s, 1H, sulfonamide), 8.24 (s, 1H, pyrazine), 8.15 (s, 1H, pyrazine), 7.04 (s, 2H, arom.), 2.66 (s, 6H, -CH_3), 2.24 (s, 3H, -CH_3).

^{1}C NMR (125 MHz, DMSO-d_6) δ 156.8, 145.1, 144.9, 141.1, 137.2, 135.1, 134.9, 130.4, 22.6, 21.9.

Elemental analysis found: C, 51.96%; H, 5.13%; N, 12.21%; S, 9.59%. **Calculated for $C_{13}H_{14}ClN_3O_2S$ (MW 311.05):** C, 50.08%; H, 4.53%; N, 13.48%; S, 10.28%.

*Yields are expressed as percentages of theoretical yields and refer to the isolated products (chromatographically pure) after all purification steps.

4.3 Biological Assays

4.3.1 In Vitro Activity Evaluation Against *Mycobacterium tuberculosis, Mycobacterium kansasii,* and *Mycobacterium avium*

Microdilution panel method. Tested strains *M. tuberculosis* H37Rv CNCTC My 331/88 (ATCC 27294), *M. kansasii* Hauduroy CNCTC My 235/80 (ATCC 12478), *M. avium* ssp. *Avium* Chester CNCTC My 80/72 (ATCC 15769) were obtained from Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague, Czech Republic. Middlebrook 7H9 broth (Sigma-Aldrich) enriched with 0.4% (*v/v*) of glycerol (Sigma-Aldrich) and 10% (*v/v*) of OADC supplement (oleic acid, albumin, dextrose, catalase; Himedia, Mumbai, India) of declared pH = 6.6. Tested compounds were dissolved and diluted in DMSO, mixed with broth (25 µL) of DMSO solution in 4.475 mL of broth and placed (100 µL) into microplate wells. Mycobacterial inocula were suspended in isotonic saline solution and the density was adjusted to 0.5–1.0 McFarland scale. These suspensions were diluted by 10^{-1} and used to inoculate the testing wells, adding 100 µL of mycobacterial suspension per well. Final concentrations of the tested compounds in wells were 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 µg/mL.

INH and PZA were used as positive controls (inhibition of growth). Negative control (mycobacterial growth control) consisted of broth plus DMSO. Plates were statically incubated in a dark, humid atmosphere at 37 ºC. After five days of incubation, 30 µL of Alamar Blue working solution (1:1 mixture of 0.1% resazurin sodium salt (aq. sol.) and 10% Tween 80) was added per well. Results were then determined after 24 h of incubation and interpreted according to Franzblau et al.[28]. The minimum inhibition concentration (MIC, µg/mL) was determined as the lowest concentration that prevented the blue to pink colour change as indicated by visual inspection. The experiments were conducted in duplicates. For the results to be valid, the difference in MIC for one compound determined from two parallel measurements must not be greater than one step on the dilution scale.

4.3.2 In Vitro Activity Evaluation Against *Mycobacterium smegmatis* and *Mycobacterium aurum*

Antimycobacterial assay was performed on fast growing *M. smegmatis* DSM 43465 (ATCC 607) and *M. aurum* DSM 43999 (ATCC 23366) from German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The technique used for activity determination was microdilution broth panel method using 96-well microtitration plates. Culturing medium was Middlebrook 7H9 broth (Sigma-Aldrich) enriched with 0.4% of glycerol (Sigma-Aldrich) and

10% of Middlebrook OADC growth supplement (Himedia). Mycobacterial strains were cultured on Middlebrook 7H9 agar and suspensions were prepared in Middlebrook 7H9 broth. Final density was adjusted to value ranging from 0.5 to 1.0 according to McFarland scale and diluted in ratio 1:20 with broth. Tested compounds were dissolved in DMSO (Sigma-Aldrich) then MB broth was added to obtain concentration of 2000 μg/mL. Standards used for activity determination were INH, rifampicin (RIF) and ciprofloxacin (CPX) (Sigma-Aldrich). Final concentrations were reached by binary dilution and addition of mycobacterial suspension, and were set as 500, 250, 125, 62.5, 31.25, 15.625, 7.81, 3.91 μg/mL, except to standards rifampicin, where the final concentrations were 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.098 μg/mL, and ciprofloxacin, where the final concentrations were 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156, 0.0078 μg/mL. The final concentration of DMSO did not exceeded 2.5% (v/v) and did not affect the growth of *M. smegmatis* or *M. aurum*. Positive (broth, DMSO, bacteria) and negative (broth, DMSO) controls were included. Plates were sealed with polyester adhesive film and incubated in dark at 37 ºC without agitation. The addition of 0.01% solution of resazurin sodium salt followed after 48 h of incubation for *M. smegmatis*, and after 72 h of incubation for *M. aurum*. Stain was prepared by dissolving resazurin sodium salt (Sigma-Aldrich) in deionised water to get 0.02% solution. Then 10% aqueous solution of Tween 80 (Sigma-Aldrich) was prepared. Equal volumes of both liquids were mixed and filtered a through syringe membrane filter. Microtitration panels were then incubated for further 2.5 h for determination of activity against *M. smegmatis*, and 4 h for *M. aurum*. Antimycobacterial activity was expressed as minimal inhibition concentration (MIC) and the value was read on the basis of stain colour change (blue colour—active compound; pink colour—inactive compound). MIC values for standards were in ranges 7.81–15.625 μg/mL for INH, 12.5–25 μg/mL for RIF, and 0.0625–0.125 μg/mL for CPX against *M. smegmatis*, 1.95–3.91 μg/mL for INH, 0.78–1.56 μg/mL for RIF, and 0.00781–0.01563 μg/mL for CPX against *M. aurum*, respectively. All experiments were conducted in duplicate. For the results to be valid, the difference in MIC for one compound determined from two parallel measurements must not be greater than one step on the dilution scale.

4.3.3 In Vitro Antibacterial Activity Evaluation

Microdilution broth method was used [29]. Antibacterial evaluation was performed against eight bacterial strains from the Czech Collection of Microorganisms (CCM, Brno, Czech Republic) (*Staphylococcus aureus* CCM 4223 (ATCC 29213), *Staphylococcus aureus* methicilin resistant CCM 4750 (ATCC 43300), *Enterococcus faecalis* CCM 4224 (ATCC 29212), *Escherichia coli* CCM

3954 (ATCC 25922), *Pseudomonas aeruginosa* CCM 3955 (ATCC 27853)) or clinical isolates from the Department of Clinical Microbiology, University Hospital and Faculty of Medicine in Hradec Králové, Charles University in Prague, Czech Republic (*Staphylococcus epidermidis* 112-2016, *Klebsiella pneumoniae* 64-2016, *Serratia marcescens* 62-2016). All strains were subcultured on Mueller-Hinton agar (MHA) (Difco/Becton Dickinson, Detroit, MI, USA) at 35 ºC and maintained on the same medium at 4 ºC. The compounds were dissolved in DMSO, and the antibacterial activity was determined in cation adjusted Mueller-Hinton liquid broth (Difco/Becton Dickinson) buffered to pH 7.0. Controls consisted of medium and DMSO solely. The final concentration of DMSO in the test medium did not exceed 1% (*v/v*) of the total solution composition. The minimum inhibitory concentration (MIC) was determined after 24 and 48 h of static incubation at 35 ºC by visual inspection or using Alamar Blue dye. The standards were gentamicin and ciprofloxacin. All experiments were conducted in duplicate. For the results to be valid, the difference in MIC for one compound determined from two parallel measurements must not be greater than one step on the dilution scale.

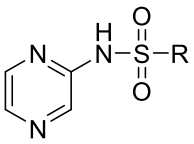
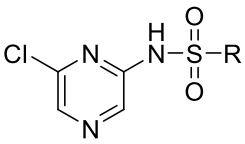
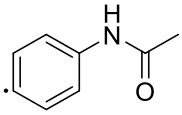
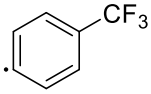
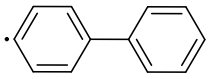
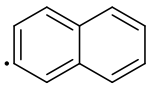
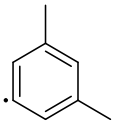
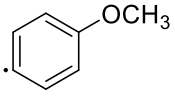
4.3.4 In Vitro Antifungal Activity Evaluation

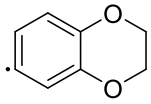
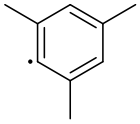
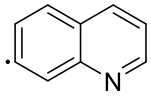
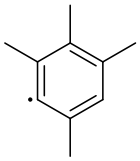
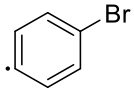
Antifungal evaluation was performed using a microdilution broth method[30] against eight fungal strains from the Czech Collection of Microorganisms (CCM) (*Candida albicans* CCM 8320 (ATCC 24433), *C. krusei* CCM 8271 (ATCC 6258), *C. parapsilosis* CCM 8260 (ATCC 22019), *C. tropicalis* CCM 8264 (ATCC 750), *Aspergillus flavus* CCM 8363, *Absidia/Lichtheimia corymbifera* CCM 8077 and *Trichophyton interdigitale* CCM 8377 (ATCC 9533) or the American Type Collection Cultures (ATCC, *Mannasas*, VA, USA) (*Aspergillus fumigatus* ATCC 204305). Compounds were dissolved in DMSO and diluted in a twofold manner with RPMI 1640 medium, with glutamine and 2% glucose, buffered to pH 7.0 (3-morpholinopropane-1-sulfonic acid). The final concentration of DMSO in the tested medium did not exceed 2.5% (*v/v*) of the total solution composition. Static incubation was performed in the dark and in humid atmosphere, at 35 ºC, for 24 and 48 h (72 and 120 h for Trichophyton interdigitale respectively). Drug-free controls were included. MIC was inspected visually or making use of Alamar Blue staining. The standards were amphotericin B and fluconazole. All experiments were conducted in duplicate. For the results to be valid, the difference in MIC for one compound determined from two parallel measurements must not be greater than one step on the dilution scale.

4.4 Results

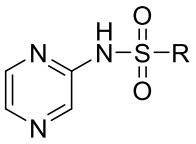
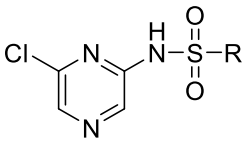
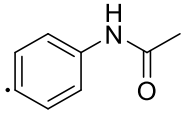
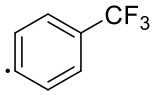
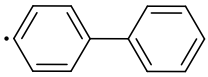
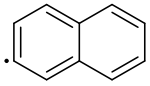
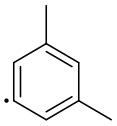
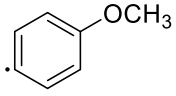
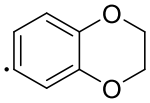
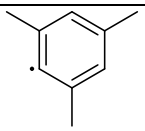
The biological results of the full series including my eight compounds are summarized below in the following tables. However, some results are missing due to uncompleted biological evaluation during my short Erasmus stay.

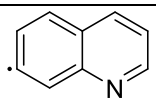
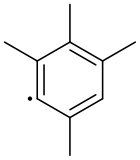
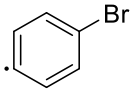
4.4.1 Antimycobacterial Activity Against *Mycobacterium tuberculosis, Mycobacterium kansasii* and *Mycobacterium avium.*

a		**b**		
				
No.	R (the dot represents the attachment point)	Antimycobacterial Activity MIC (µg/mL)		
		Mtb	*M. Kansasii*	*M. avium*
1a		NA	NA	NA
1b		NA	NA	NA
2a		>100	**25**	>100
2b		NA	NA	NA
3a		>100	50	>100
3b		NA	NA	NA
4a		100	100	>100
4b		100	**12.5**	>100
5a		>100	50	>100
5b		50	50	>100
6a		>100	50	>100
6b		NA	NA	NA
7a		>100	>100	>100
7b		>100	100	>100

8a		NA	NA	NA
8b		NA	NA	NA
9a		>100	>100	>100
9b		NA	NA	NA
10a		NA	NA	NA
11a		>100	50	>100
12b		100	50	>100
PZA	----------	>100	>100	>100
INH	----------	0.2	25	12.5

4.4.2 Antimycobacterial Activity Against *Mycobacterium smegmatis, Mycobacterium aurum.*

a		b	
			
No.	R (the dot represents the attachment point)	Antimycobacterial Activity MIC (µg/mL)	
		M. smegmatis	*M. aurum*
1a		**≥250**	≥500
1b		**250**	≥500
2a		≥500	≥500
2b		**250**	≥500
3a		**250**	**250**
3b		**250**	**250**
4a		≥500	≥500
4b		≥500	≥500
5a		**62.5**	≥500
5b		**125**	**250**
6a		≥500	**250**
6b		NA	NA
7a		≥500	≥500
7b		NA	NA
8a		≥500	**250**
8b		NA	NA
9a		**125**	≥500
9b		NA	NA

10a		≥500	≥500
11a		≥500	≥500
12b		**125**	**125**
INH	----------	15.625	3.91
RFM	----------	25	1.56
CPX	----------	0.125	0.008

4.4.3 Antibacterial Assay Results of Prepared Compounds.

Pathogen	Time(h)	Antibacterial Activity [MIC (μmol.l^{-1})]														
		1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a	7b	8a
SA	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
MRSA	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
SE	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
EF	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
EC	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
KP	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
SEMA	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
PA	72hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	120hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500

1. **SA**- *Staphylococcus aureus*
2. **MRSA**- *Staphylococcus aureus* methicilin resistant
3. **SE**- *Staphylococcus epidermidis*
4. **EF**- *Enterococcus faecalis*
5. **EC**- *Escherichia coli*
6. **KP**- *Klebsiella pneumoniae*
7. **SEMA**- *Serratia marcescens*
8. **PA**- *Pseudomonas aeruginosa*

Pathogen	Time(h)	Antibacterial Activity [MIC (μmol.l^{-1})]					
		8b	9a	9b	10a	11a	12b
SA	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
MRSA	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
SE	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
EF	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
EC	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
KP	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
SEMA	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
PA	72hr	>500	>500	>500	>500	>500	>500
	120hr	>500	>500	>500	>500	>500	>500

1. **SA**- *Staphylococcus aureus*
2. **MRSA**- *Staphylococcus aureus* methicilin resistant
3. **SE**- *Staphylococcus epidermidis*
4. **EF**- *Enterococcus faecalis*
5. **EC**- *Escherichia coli*
6. **KP**- *Klebsiella pneumoniae*
7. **SEMA**- *Serratia marcescens*
8. **PA**- *Pseudomonas aeruginosa*

4.4.4 Antifungal Assay Results of Prepared Compounds.

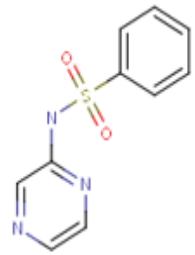
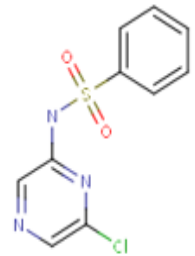
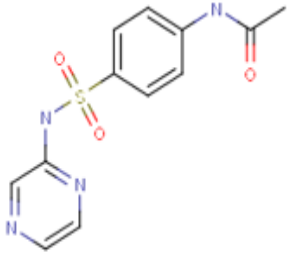
Pathogen	Time(h)	Antifungal Activity [MIC (μmol.l^{-1})]															
		1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a	7b	8a
CA	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
CK	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
CP	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
CT	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
AF	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
AFla	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
AC	24hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
TI	72hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	120hr	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500

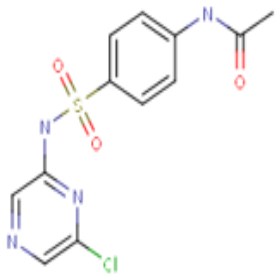
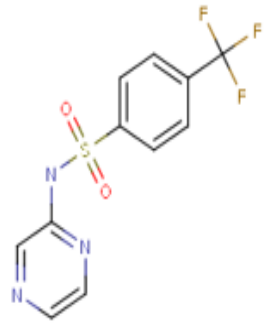
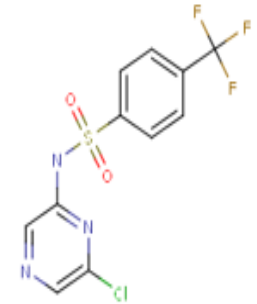
1. **CA1**- *Candida albicans*
2. **CK**- *Candida krusei*
3. **CP**- *Candida parapsilosis*
4. **CT**- *Candida tropicalis*
5. **AF**- *Aspergillus fumigatus*
6. **AFla**- *Aspergillus flavus*

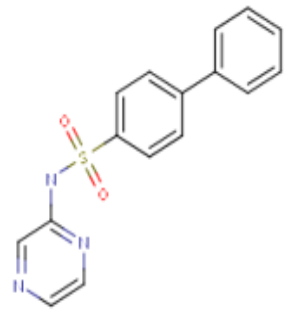
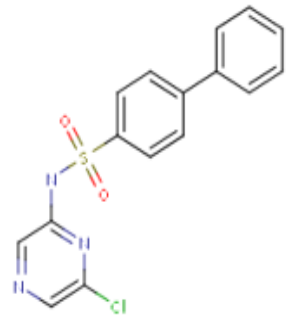
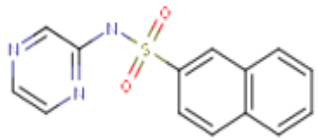
Pathogen	Time(h)	Antifungal Activity [MIC (µmol.l^{-1})]					
		8b	9a	9b	10a	11a	12b
SA	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
MRSA	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
SE	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
EF	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
EC	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
KP	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
SEMA	24hr	>500	>500	>500	>500	>500	>500
	48hr	>500	>500	>500	>500	>500	>500
PA	72hr	>500	>500	>500	>500	>500	>500
	120hr	>500	>500	>500	>500	>500	>500

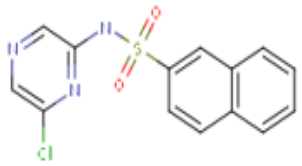
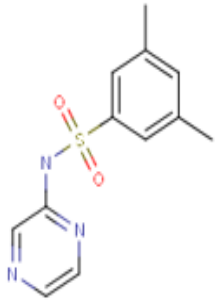
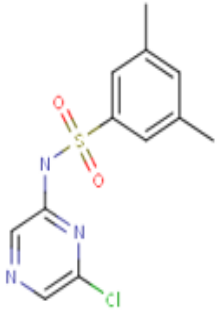
1. **CA1**- *Candida albicans*
2. **CK**- *Candida krusei*
3. **CP**- *Candida parapsilosis*
4. **CT**- *Candida tropicalis*
5. **AF**- *Aspergillus fumigatus*
6. **AFla**- *Aspergillus flavus*
7. **AC**- *Absidia/Lichtheimia corymbifera*
8. **TI**- *Trichophyton interdigitale*

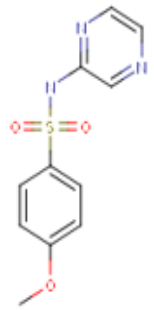
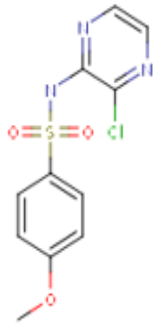
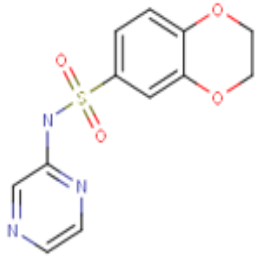
5. DESCRPITORS OF PREPARED COMPOUNDS.

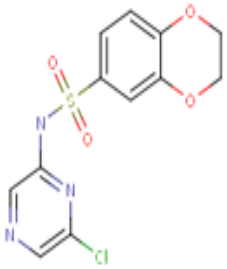
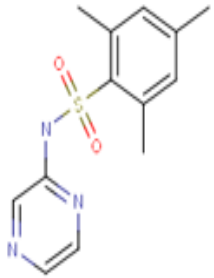
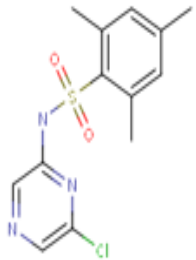
STRUCTURE	Code	Heavy atoms	Rotatable bonds	HBD	HBA	cLogP	cLogD	TPSA	pKa	Ro5	Ro3	Lead likeness
	1a	16	2	1	4	0,620024	-0,18212	71,95	6,114729	1	0	1
	1b	17	2	1	4	1,444246	0,62532	71,95	6,047709	1	0	1
	2a	20	3	2	5	-0,14227	-0,93072	101,05	6,164846	1	0	1

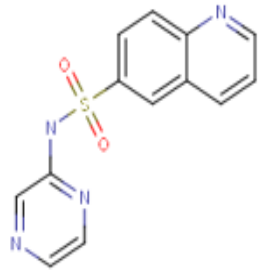
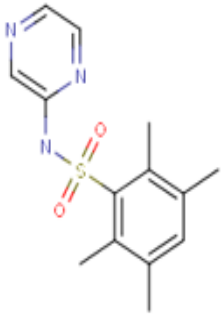
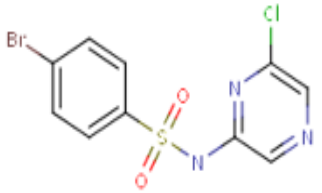
STRUCTURE	Code	Heavy atoms	Rotatable bonds	HBD	HBA	cLogP	cLogD	TPSA	pKa	Ro5	Ro3	Lead likeness
	2b	21	3	2	5	0,681956	-0,12571	101,05	6,093402	1	0	1
	3a	20	3	1	4	1,497872	0,691862	71,95	6,099865	1	0	1
	3b	21	3	1	4	2,322094	1,499972	71,95	6,034125	1	0	1

STRUCTURE	Code	Heavy atoms	Rotatable bonds	HBD	HBA	cLogP	cLogD	TPSA	pKa	Ro5	Ro3	Lead likeness
	4a	22	3	1	4	2,267249	1,465034	71,95	6,114442	1	0	1
	4b	23	3	1	4	3,091471	2,272483	71,95	6,047447	1	0	1
	5a	20	2	1	4	1,609501	0,795964	71,95	6,06998	1	0	1

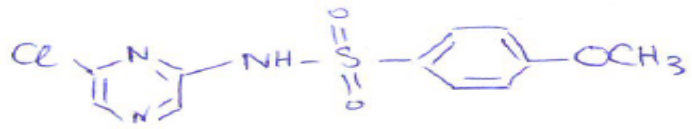
STRUCTURE	Code	Heavy atoms	Rotatable bonds	HBD	HBA	cLogP	cLogD	TPSA	pKa	Ro5	Ro3	Lead likeness
	5b	21	2	1	4	2,433722	1,605367	71,95	6,006768	1	0	1
	6a	18	2	1	4	1,646867	0,845542	71,95	6,117821	1	0	1
	6b	19	2	1	4	2,471088	1,652836	71,95	6,050534	1	0	1

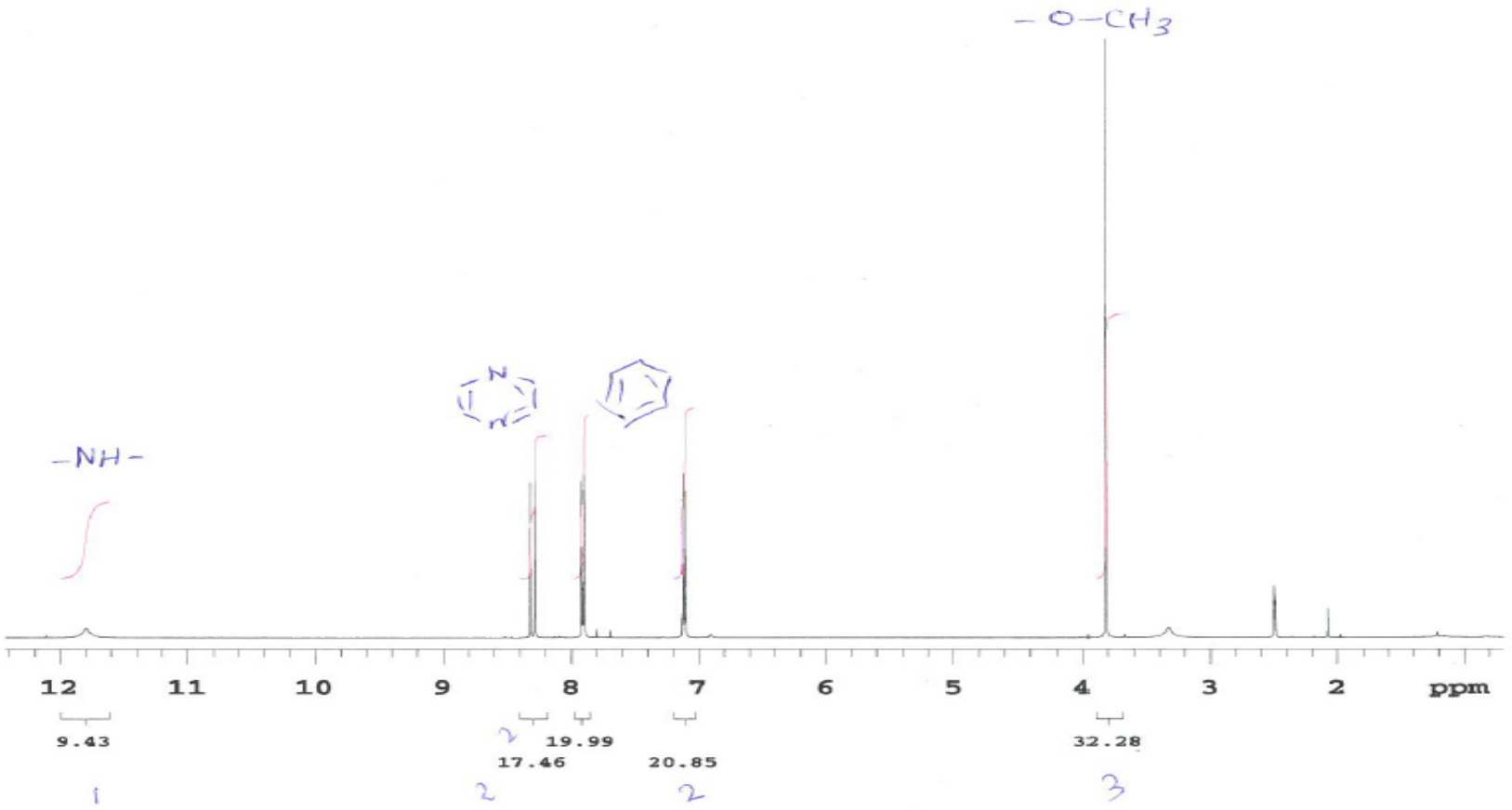
STRUCTURE	Code	Heavy atoms	Rotatable bonds	HBD	HBA	cLogP	cLogD	TPSA	pKa	Ro5	Ro3	Lead likeness
	7a	18	3	1	5	0,462353	-0,28506	81,18	6,297503	1	0	1
	7b	19	3	1	5	1,286574	1,094784	81,18	7,570751	1	0	1
	8a	20	2	1	6	0,133156	-0,65759	90,41	6,156723	1	0	1

STRUCTURE	Code	Heavy atoms	Rotatable bonds	HBD	HBA	cLogP	cLogD	TPSA	pKa	Ro5	Ro3	Lead likeness
	8b	21	2	1	6	0,957378	0,147836	90,41	6,086009	1	0	1
	9a	19	2	1	4	2,160288	1,444296	71,95	6,38637	1	0	1
	9b	20	2	1	4	2,98451	2,235678	71,95	6,293281	1	0	1

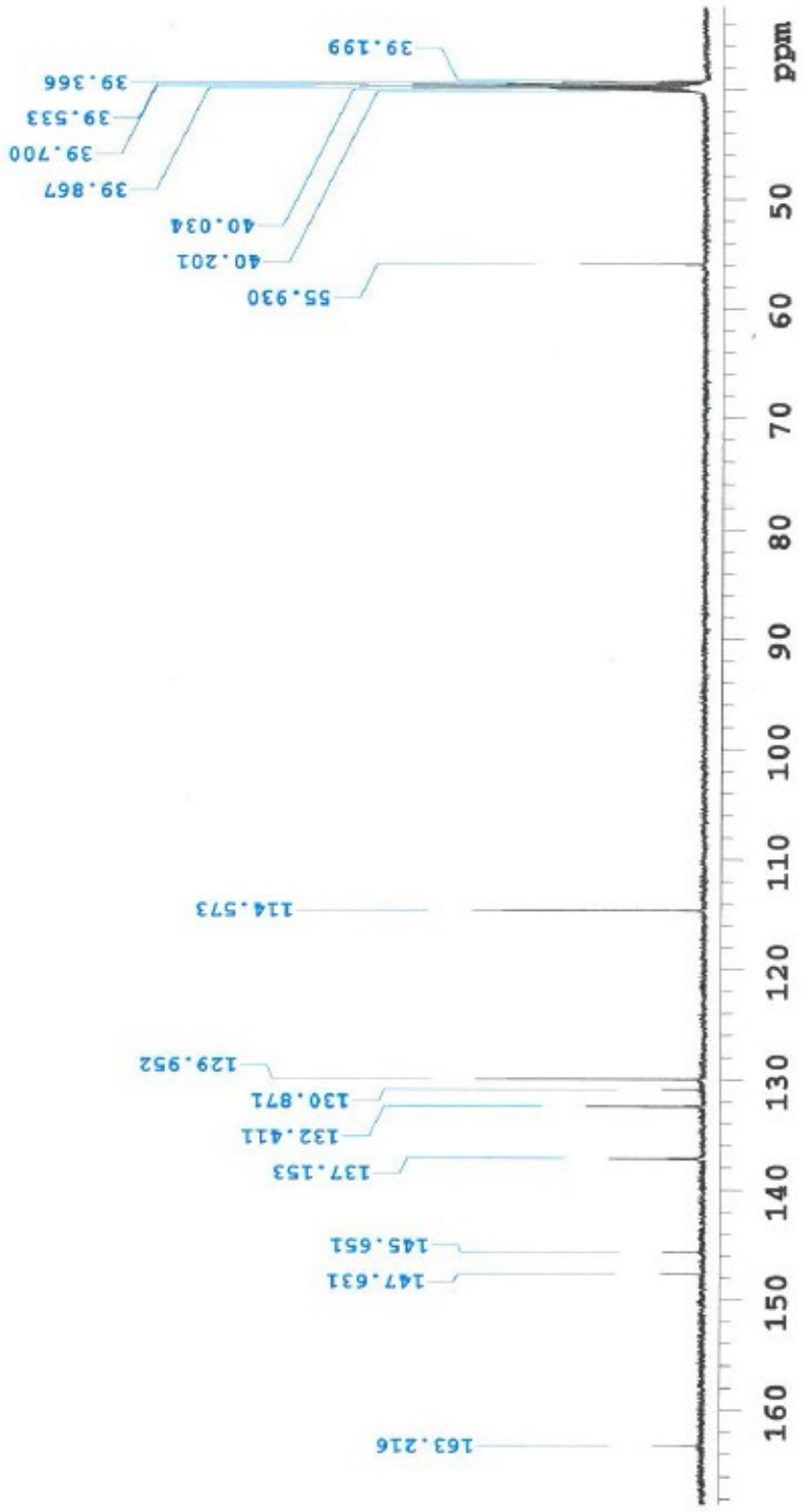
STRUCTURE	Code	Heavy atoms	Rotatable bonds	HBD	HBA	cLogP	cLogD	TPSA	pKa	Ro5	Ro3	Lead likeness
	10a	20	2	1	5	0,777678	-0,01768	84,84	6,144764	1	0	1
	11a	20	2	1	4	2,673709	1,909968	71,95	6,247416	1	0	1
	12b	18	2	1	4	2,212998	1,390296	71,95	6,031629	1	0	1

6.1 [1]HNMR Spectra of Compound 7b

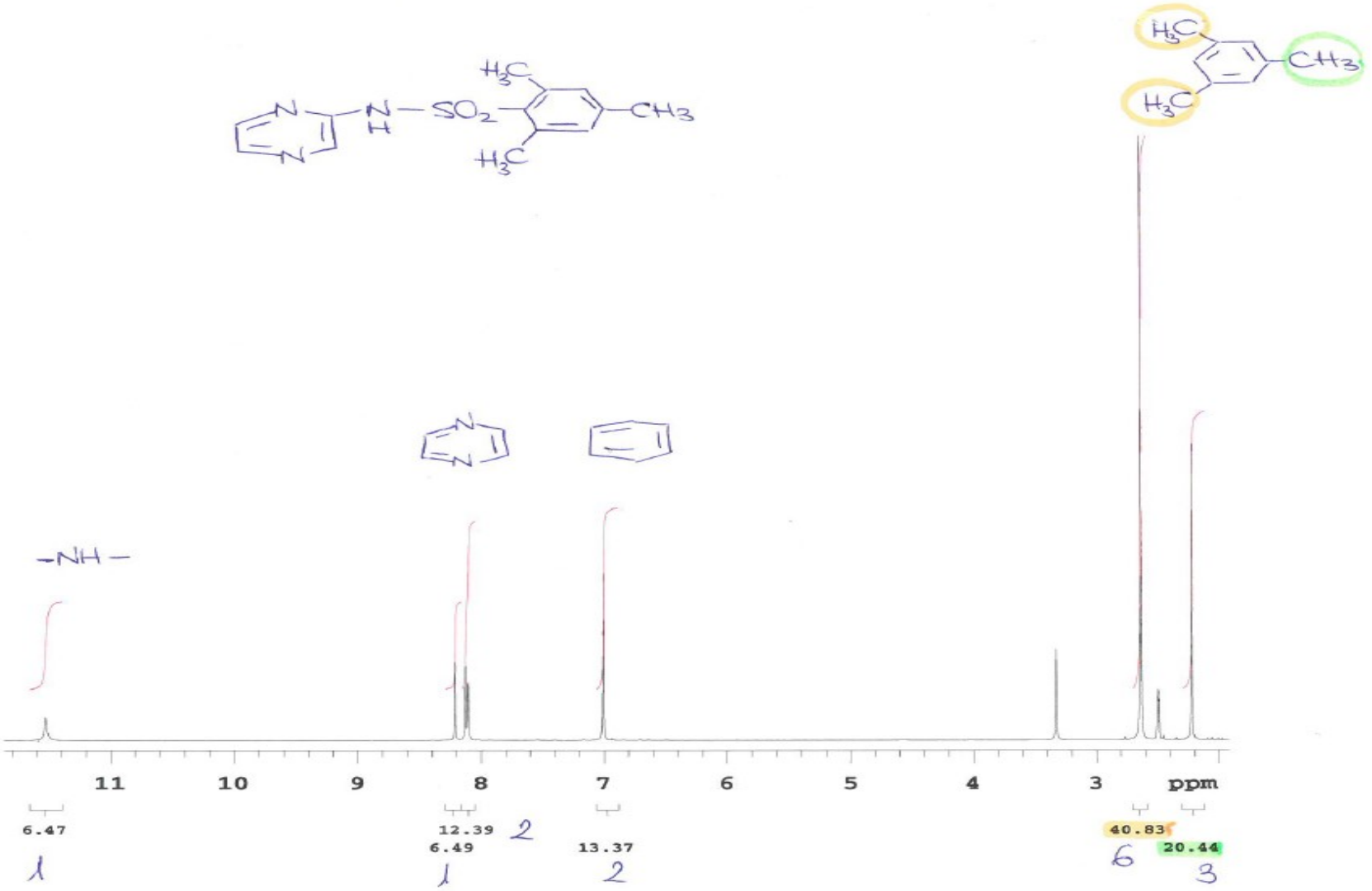




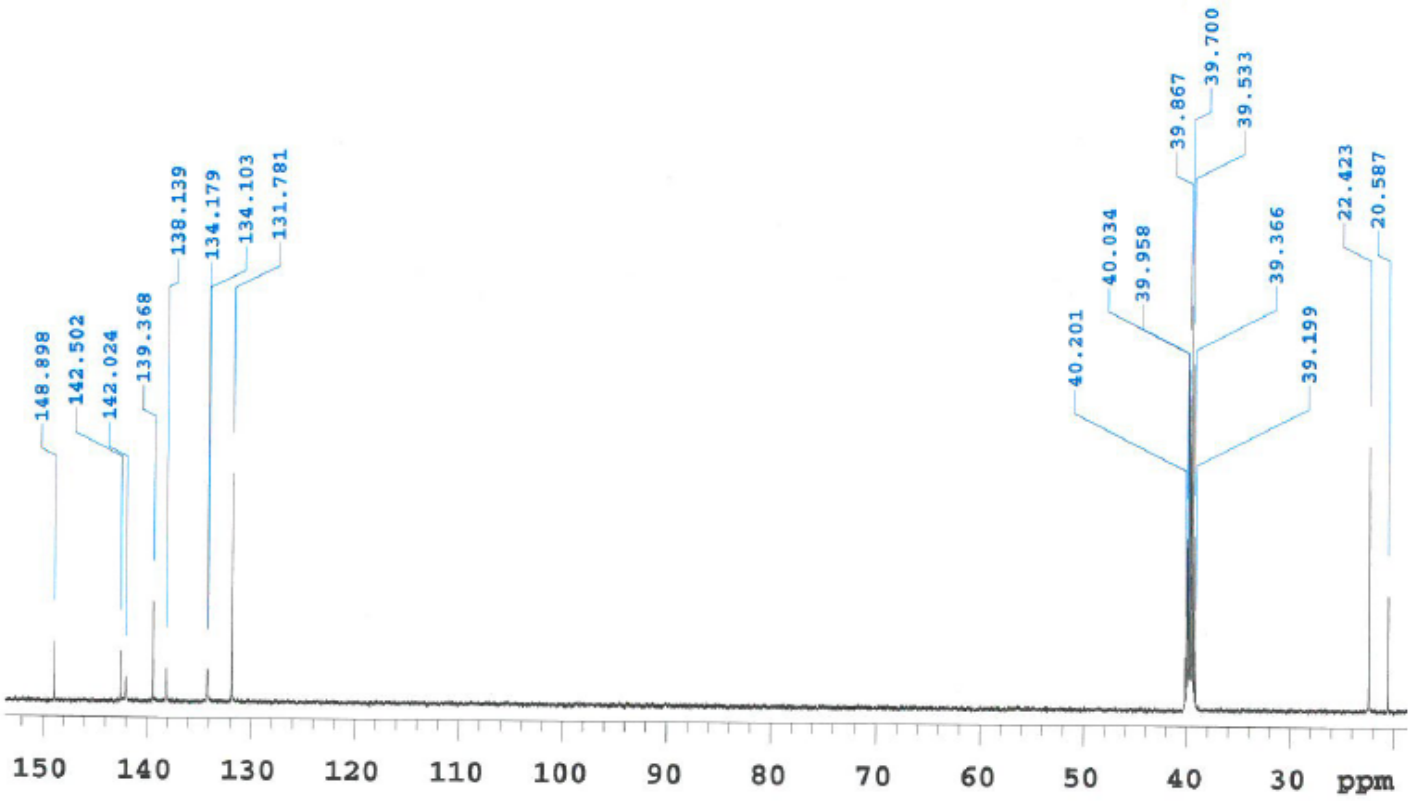
6.2 ^{13}CNMR Spectra of Compound 7b



6.3 ^{1}HNMR Spectra of Compound 9a



6.4 ^{13}CNMR Spectra of Compound 9a



6. DISCUSSION

All synthesized compounds, were evaluated for *in vitro* activity against *Mtb* H37R$_v$, *M. Kansasii* and *M. avium* using a Microplate Alamar Blue Assay. Antimycobacterial activity results were expressed as minimum inhibitory concentration (MIC) in µg·mL^{-1} against INH as standard. Based on the so far available data, two compounds, **2a** (MIC $_{M.\ Kansasii}$ = 25 µg/mL) and **4b** (MIC $_{M.\ Kansasii}$ = 12.5 µg/mL) (**Figure 7**), had activity against *M. kansasii*. For compound **2a** particularly, this activity is justifiable from its chemical structure. The acetamide moiety in the structure may have worked as a pro-drug that was hydrolyzed by *M. Kansasii* amidase to the free amino group (NH_2-)[31, 32]. A free amino group is needed for the anti-infective activity of sulfonamides[33]. The activity against *M. kansasii* and not against any of the other mycobacterial strains may be attributed to the different substrate specifity of mycobacterial amidases among different mycobacterial strains[31, 32]. Furthermore, sulfametopyrazine is an established drug with antibacterial activity that has a chemical structure closely related to the proposed structure of the active form of compound **2a** (**Figure 7**)[34]**.**

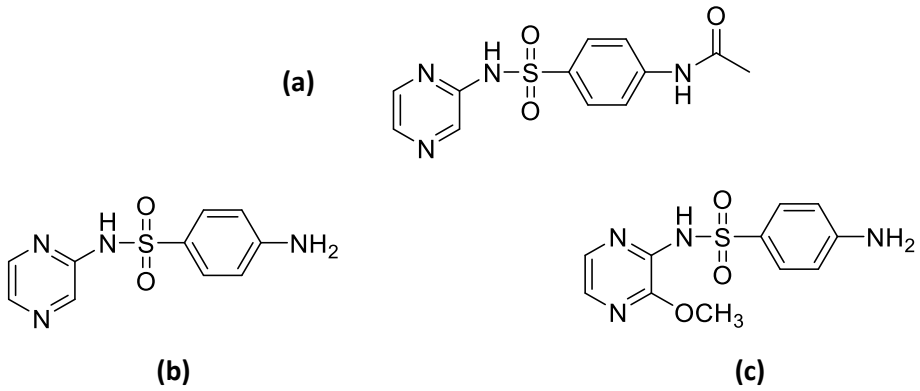


Figure 7: (a) Chemical structure of compound 2a (b) The proposed chemical structure of the active form of compound 2a (c) The chemical structure of sulfametopyrazine.

The full series was also evaluated for antimycobacterial activity against the two fast growing mycobacterial strains, *M. smegmatis* and *M. aurum*. Being a virulent surrogate organisms with better safety profiles and short replication time, make those two strains advantageous over the conventional *Mtb*. Microplate Alamar Blue Assay was used for this test with INH, rifampicin, and ciprofloxacin as standards. Some compounds exerted moderate activity against the two tested strains (corresponding values are **in bold** on pages 34 and 35).

All prepared compounds were screened *in vitro* for biological activity against eight common bacterial strains and eight fungal stems of clinical importance using standard methods. No antibacterial or antifungal activities were observed for any of the tested compounds up to the highest tested concentrations.

The descriptors of prepared compounds show that all comply with the rule of 5 and are lead-like compounds.

7. CONCLUSIONS

To conclude, a series of 22 different substituted *N*-(pyrazin-2-yl)benzenesulfonamides was designed and synthesized. During my short Erasmus stay, I have contributed to the synthesis and purification of 8 different compounds from this series. The prepared compounds were then evaluated for their anti-infective activity against *Mycobacterium tuberculosis* and four other nontubercular mycobacterial strains, along with antibacterial and antifungal activity evaluation.

According to the results of antimycobacterial assays, none of the compounds showed activity against any of the mycobacterial strains except for two compounds. Compounds **2a** and **4b** had activity against *Mycobacterium kansasii* but not against any other, suggesting that these two compounds have higher affinity to *Mycobacterium kansasii* target enzyme and/or they two exert their antimycobacterial activity through a certain pathway not shared among all mycobacterial strains.

As mentioned earlier in the introduction, the chemical structure of zibotentan is similar to the chemical structure of our prepared compounds, and thus our compounds will be evaluated for their *in vitro* cytotoxicity against HepG2 liver cancer cell line.

Additionally, our compounds will be evaluated by The Lilly Open Innovation Drug Discovery Program (OIDD) provided by the American company Eli Lilly, in which such molecules are tested in context of metabolic disorders such as diabetes, osteoporosis and dyslipidemia.

8. ABSTRAKT (CZECH)

Univerzita Karlova

Farmaceutická fakulta v Hradci Králové

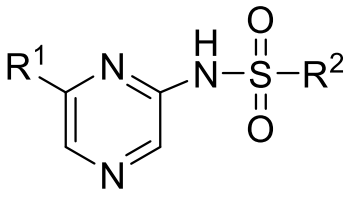
Katedra farmaceutické chemie a farmaceutické analýzy

Řešitel: Cristina Paredes de la Red

Vedoucí diplomové práce: prof. PharmDr. Martin Doležal, Ph.D.

Název diplomové práce: Syntéza a antiinfekční hodnocení substituovaných N-(pyrazin-2-yl)benzensulfonamidů

Tuberkulóza (TBC) patří celosvětově mezi deset nejčastějších příčin úmrtí, zejména v rozvojových zemích. I když se jedná o dlouho známé onemocnění se zavedeným léčebným režimem, vyskytuje se v poslední době zvýšená rezistence na léčiva proti TBC. Díky novým poznatkům a teoriím o jeho mechanismu účinku se antituberkulotikum první linie pyrazinamid opět dostává do popředí vědeckého zájmu a stává se perspektivní výchozí sloučeninou pro další vývoj. V této práci prezentujeme *N*-(pyrazin-2-yl) benzensulfonamidy (obecná struktura je uveden na obrázku níže) jako nové hybridní molekuly spojující strukturu pyrazinamidu a sulfonamidu. Antibakteriální sulfonamidy účinkují mechanismem kompetitivní inhibice syntézy kyseliny listové s následnou inhibicí bakteriálního růstu a rozmnožování. Osobně jsem v rámci této práce přispěla k syntéze a čištění osmi finálních sloučenin v sérii celkem 22 *N*-pyrazinylsulfonamidů. Dvě z připravených sloučenin vykázaly aktivitu proti Mycobacterium kansasii [**2a** (MIC $_{M.\ Kansasii}$ = 25 µg/mL); **4b** (MIC $_{M.\ Kansasii}$ = 12.5 µg/mL)]. Některé sloučeniny vykázaly mírnou aktivitu proti *Mycobacterium aurum* a *Mycobacterium smegmatis*.



R^1 : H, Cl; R^2 : Aromatic substituents

9. ABSTRACT (ENGLISH)

Charles University

Faculty of Pharmacy in Hradec Králové

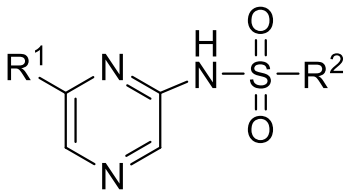
Department of Pharmaceutical Chemistry and Pharmaceutical Analysis

Author: Cristina Paredes de la Red

Supervisor: prof. PharmDr. Martin Doležal, Ph.D.

Title of diploma thesis: Synthesis and antiinfective evaluation of substituted *N*-(pyrazine-2-yl)benzenesulfonamide

Tuberculosis (TB) is among the ten leading causes of death, especially in developing countries. Even though it is an old disease with established treatment regimen, there has been an increased resistance to anti-TB drugs [1]. The anti-tubercular pyrazinamide has caught the attention of researchers as the different theories for its mechanism of action have made it an interesting entity for further investigation. Here we will discuss *N*-(pyrazin*e*-2-yl)benzenesulfonamides (General structure is presented in the Figure below) as a new derivatization approach based on synergism methodology between pyrazinamide and sulfonamides. Sulfonamides exert their antimicrobial effect by competitive inhibition of folic acid synthesis and subsequent inhibition of bacterial growth and reproduction [18]. I have contributed to the synthesis and purification of 8 compounds in a series of total 22 *N-pyrazinylsulfonamides*. Two of the prepared compounds showed activity against *Mycobacterium kansasii* [**2a** (MIC $_{M.\ Kansasii}$ = 25 μg/mL); **4b** (MIC $_{M.\ Kansasii}$ = 12.5 μg/mL)]. Some compounds showed a moderate activity against *Mycobacterium aurum* and *Mycobacterium smegmatis*.



R^1 : H, Cl; R^2 : Aromatic substituents

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