

CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ
DPT. OF PHARMACEUTICAL CHEMISTRY AND PHARMACEUTICAL
ANALYSIS



DERIVATIVES OF QUINOXALINE-2-CARBOXYLIC ACID AS
POTENTIAL ANTIMICROBIAL COMPOUNDS

Diploma Thesis

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Supervised by: Jan Zitko, Ph.D.

Hradec Králové, 2019

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“I declare that this thesis is my original author's work, which has been composed solely by myself (under the guidance of my consultant, Dr. Jan Zitko). All the literature and other resources from which I drew information are cited in the list of used literature and are quoted in the paper. The work has not been used to get another or the same title.”

Sarah Bouz

Hradec Králové, 15th of May of 2019

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1. LIST OF ABBREVIATIONS

BCG	Bacillus Calmette–Guérin
CoA	Coenzyme A
CPX	Ciprofloxacin
DCM	Dichloromethane
DMF	<i>N,N</i> -dimethylformamide
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl acetate
FAS I	Fatty acid synthase I
FDA	Food and drug administration
HIV	Human immunodeficiency virus
IC ₅₀	Half maximal inhibitory concentration
INH	Isoniazid
IR	Infrared
MDR-TB	Multidrug-resistant tuberculosis
MIC	Minimum inhibition concentration
<i>Mtb</i>	<i>Mycobacterium tuberculosis</i>
QAPRTase	Quinolinic acid phosphoribosyltransferase
NA	Not available
NAD	Nicotinamide adenine dinucleotide

NMR	Nuclear magnetic resonance
OADC	Oleic acid, albumin, dextrose, catalase
PABA	<i>Para</i> -aminobenzoic acid
RFM	Rifampicin
RpsA	Ribosomal protein S1
PanD	Aspartate decarboxylase
POA	Pyrazinoic acid
PZA	Pyrazinamide
QA	Quinolinic acid
RIF	Rifampicin
SI	Selectivity index
TB	Tuberculosis
TLC	Thin layer chromatography
UV	Ultra violet
XDR-TB	Extensively drug-resistant tuberculosis

2. ABSTRAKT (CZECH)

Univerzita Karlova

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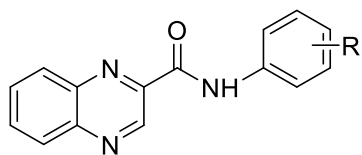
Katedra farmaceutické chemie a farmaceutické analýzy

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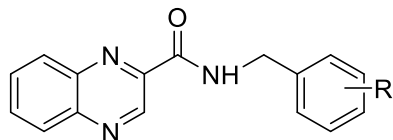
Školitel: PharmDr. Jan Zítka, Ph.D.

Název diplomové práce: Deriváty chinoxalin-2-karboxylové kyseliny jako potenciální antimikrobní látky

I přes dostupnost prověřených léčebných režimů zůstává tuberkulóza nejčastější příčinou úmrtí mezi infekčními nemocemi. Jednou z příčin selhání snah o eradikaci této nemoci je léková rezistence. Tato skutečnost zvyšuje celosvětové úsilí ve vývoji nových antituberkulotik. V rámci našeho dlouhodobého výzkumu derivátů pyrazinu jsme připravili sérii *N*-substituovaných chinoxalin-2-karboxamidů, viz obr. níže. Chinoxalin-2-karboxylová kyselina byla aktivována působením oxalyl chloridu a posléze reagovala s různými aniliny či benzylaminy za přítomnosti pyridinu za míchání při laboratorní teplotě přes noc. Získané surové produkty byly dále čištěny pomocí flash chromatografie. Finální produkty byly hodnoceny na *in vitro* antimikrobiální aktivitu vůči šesti mykobakteriálním kmenům, osmi kmenům hub, čtyřem gram-pozitivním a čtyřem gram-negativním bakteriálním kmenům klinického významu. Nejslibnějších výsledků a nejširšího spektra antimykobakteriálního účinku ($MIC_{MtbH37Ra} = 3,91 \mu\text{g/mL}$, $MIC_{MtbH37Rv} = 50 \mu\text{g/mL}$, $MIC_{M. kansasii} = 50 \mu\text{g/mL}$, $MIC_{M. avium} = 50 \mu\text{g/mL}$, $MIC_{M. aurum} = 125 \mu\text{g/mL}$, $MIC_{M. smegmatis} \geq 250 \mu\text{g/mL}$) bylo dosaženo u *N*-(4-methoxybenzyl)chinoxalin-2-karboxamidu (JZAS-9), který vykázal rovněž nízkou toxicitu (index selektivity účinku $SI = 16$).



R = H, 3-CF₃, 3-OH, 4-OH, 3,5-diOCH₃



R' = H, 2-Cl, 2-CH₃, 3-Cl, 3-F, 3-CF₃,
4-CH₃, 4-OCH₃, 4-OH, 2,5-diOCH₃, 3,5-diOCH₃

Obr. Chemické struktury finálních sloučenin.

3. ABSTRACT (ENGLISH)

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Faculty of Pharmacy in Hradec Králové

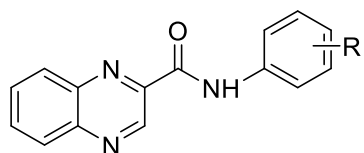
Department of Pharmaceutical Chemistry and Pharmaceutical Analysis

Candidate: Sarah Basem Bouz

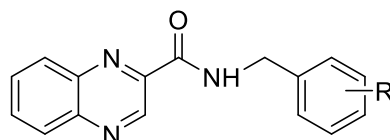
Supervisor: PharmDr. Jan Zitko, Ph.D.

Title of diploma thesis: Derivatives of quinoxaline-2-carboxylic acid as potential antimicrobial compounds

Despite the presence of well-established treatment plan, tuberculosis remains the number one killer of infections according to WHO. One of the reasons behind this failure in eradicating this infection is drug resistance. This fact potentiates worldwide efforts to develop new antituberculars. As part of our long-term research on pyrazine derivatives, we prepared a series of *N*-substituted quinoxaline-2-carboxamides, refer to fig. below. Quinoxaline-2-carboxylic acid was activated by oxalyl chloride and reacted with different anilines or benzylamines in the presence of pyridine at room temperature, overnight with stirring, and then obtained crudes were purified with flash chromatography. Final products were evaluated for *in vitro* antimicrobial activities against six mycobacterial strains, eight fungal stems, along with four gram positive and four gram negative bacteria of clinical importance. The most promising compound among all with broad spectrum of antimycobacterial activity ($MIC_{MtbH37Ra} = 3.91 \mu\text{g/mL}$, $MIC_{MtbH37Rv} = 50 \mu\text{g/mL}$, $MIC_{M. kansasii} = 50 \mu\text{g/mL}$, $MIC_{M. avium} = 50 \mu\text{g/mL}$, $MIC_{M. aurum} = 125 \mu\text{g/mL}$, $MIC_{M. smegmatis} \geq 250 \mu\text{g/mL}$) and high selectivity index ($SI = 16.1$) was *N*-(4-methoxybenzyl)quinoxaline-2-carboxamide (JZAS-9).



R = H, 3-CF₃, 3-OH, 4-OH, 3,5-diOCH₃

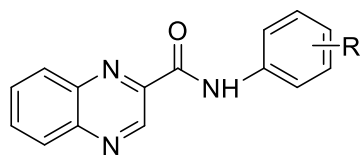


R' = H, 2-Cl, 2-CH₃, 3-Cl, 3-F, 3-CF₃,
4-CH₃, 4-OCH₃, 4-OH, 2,5-diOCH₃, 3,5-diOCH₃

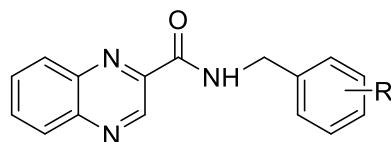
Fig. The chemical structures of title compounds.

4. AIM OF WORK

The work presented in this diploma thesis aimed at designing and synthesizing 16 different derivatives of quinoxaline-2-carboxylic acid. Title compounds were further divided into two groups based on the absence or presence of a methylene bridge linking the quinoxaline carboxamide to the aromatic substituents, see Figure below. In addition to chemical synthesis, this diploma work discusses the biological evaluation of title compounds as anti-infectives. *In vivo* biological evaluation consisted of antimycobacterial assays against 6 mycobacterial strains, antibacterial assays against 8 bacterial strains, antifungal assays against 8 fungal stems, and cytotoxicity against HepG2 liver cancer cell line. Last but not least, obtained biological results were compared to previous results of structurally related compounds prepared by our research group to draw structure activity relationship conclusions.



SB2, JZAS-3, JZAS-4, JZAS-2, SB-1



JZAS-6, JZAS-7, JZAS-8, JZAS-9, JZAS-13, JZAS-10,
JZAS-11, JZAS-14, JZAS-14, JZAS-16, JZAS-17, JZAS-15

5. INTRODUCTION

5.1. Tuberculosis Epidemiology and Pathophysiology

Tuberculosis (TB), previously known as the white death, remains the number one killer from infectious diseases despite the availability of established treatment regimen.^[1] According to World Health Organization (WHO) Global Tuberculosis Report, there are 10 million new cases of TB in 2017 alone.^[1] TB is caused by mycobacteria belonging to tuberculosis complex. These include the classical tubercle bacillus *M. tuberculosis (Mtb)*, *M. bovis*, *M. africanum*, *M. canetti*, *M. microti*, *M. pinnipedi*, and *M. caprae*.^[2] One very important characteristic of mycobacteria is the thick lipophilic mycolic cell wall.^[2] From medicinal chemistry point of view, this feature creates a challenge for drug development to design new potential antimycobacterials with sufficient lipophilicity to penetrate such thick wall. TB mostly affects the lungs, yet other organs such as the meninges and bones can also be infected.^[3] TB infection is acquired by inhaling infected droplets from a person with active TB while coughing or even talking. Several risk factors increase the probability of acquiring TB infection, for example longer duration of exposure to an infected individual and having weakened immune system either because of disease (HIV, cancer, diabetes, immunodeficiency) or medications (corticosteroids, immunosuppressants).^[4] Once *Mtb* enter the body, they are engulfed by macrophages where they slowly replicate. Other macrophages and immune cells are transferred as part of defense mechanism to the site of infection, either in the lungs (pulmonary TB) or outside the lungs (extrapulmonary TB), forming the characteristic TB granulomas.^[5] The infected individual might develop signs and symptoms of active disease, including cough, fatigue, breathless, night sweats, etc. or develop what is known as latent TB, which is the situation when the individual is infected with the bacteria but doesn't experience signs and symptoms.^[6] It is estimated that 23% of the world's population have latent TB.^[1] Active TB is highly contagious and is considered to be a public health issue. Individuals with active TB are kept in isolation until their sputum is clear from mycobacteria.^[4] On the other hand, patients with latent TB are not contagious and cannot transmit the disease. The outcome of the exposure to an individual with active TB is summarized in Figure 1.

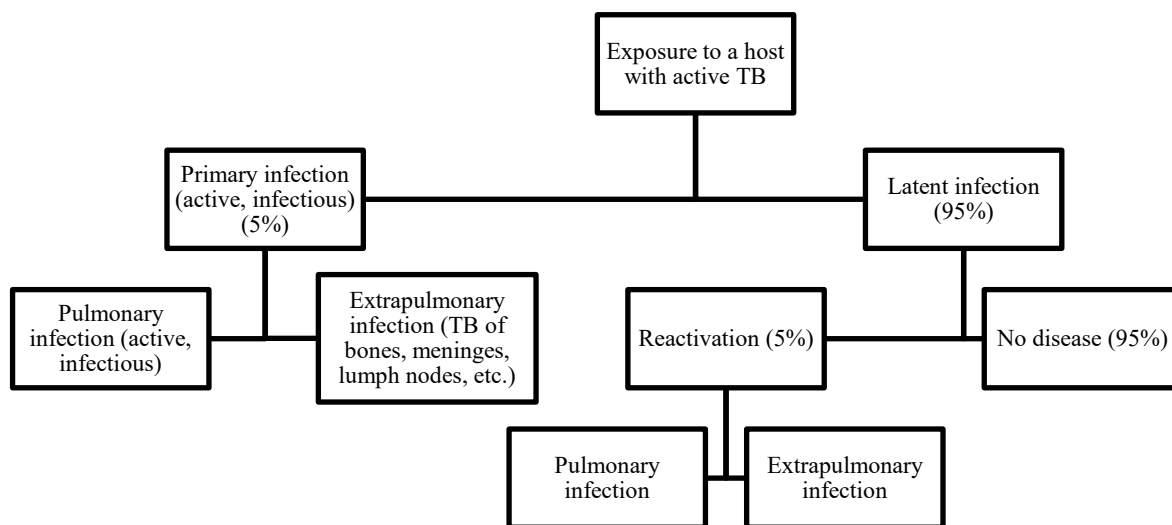


Figure 1: Outcome of the exposure to an active TB case.

5.2. Tuberculosis Prevention and Treatment

The only available vaccine against TB is Bacillus Calmette–Guérin (BCG) vaccine. Yet this vaccine is not sufficient to prevent TB infection in adults and is only effective against disseminated forms of TB in children.^[7] New vaccines against TB are currently in clinical trials.^[8] First line antituberculars include isoniazid (INH), rifampicin (RFM), pyrazinamide (PZA), and ethambutol (Figure 2). Second line agents include streptomycin, fluoroquinolones, amikacin, kanamycin, capreomycin, ethionamide, and *p*-aminosalicylic acid (Figure 2).^[4] The classical treatment of drug-sensitive TB consists of a complex regimen with 4 drugs and duration of 6 months or more; the initial phase of treatments consists of INH, RFM, PZA, and ethambutol for 2 months followed by the continuation phase that consists of INH and RFM for additional 4 months.^[4, 9] The reasons behind the incomplete eradication of this infection range from inaccessibility to treatment and poor adherence to drug resistance. Drug resistance includes multidrug-resistant TB (MDR-TB), when the bacteria is resistant to both INH and RFM, and extensively drug resistant TB (XDR-TB), when the bacteria is resistant to INH, rifampicin, fluoroquinolones, and one of the three parenteral second line drugs (amikacin, kanamycin, or capreomycin).^[10] Drug resistance is an alarming issue that requires prompt efforts to discover new agents *Mtb* are sensitive to.^[11]

FDA has recently approved two new drugs against drug resistant TB; delamanid which is an inhibitor of mycolic cell wall synthesis and bedaquiline which is an ATP synthase inhibitor.^[12, 13] Another challenge that faces TB management is the co-infection with Human Immunodeficiency Virus (HIV). HIV itself makes the infected host more susceptible to acquire infections due to immunocomprimization.^[14] Furthermore, antiviral agents used to manage HIV interact with antituberculars, especially the backbone antitubercular RFM, resulting in lowering the efficacy of the agents used for both conditions and augmenting adverse effects, hepatotoxicity particularly (Table 1).

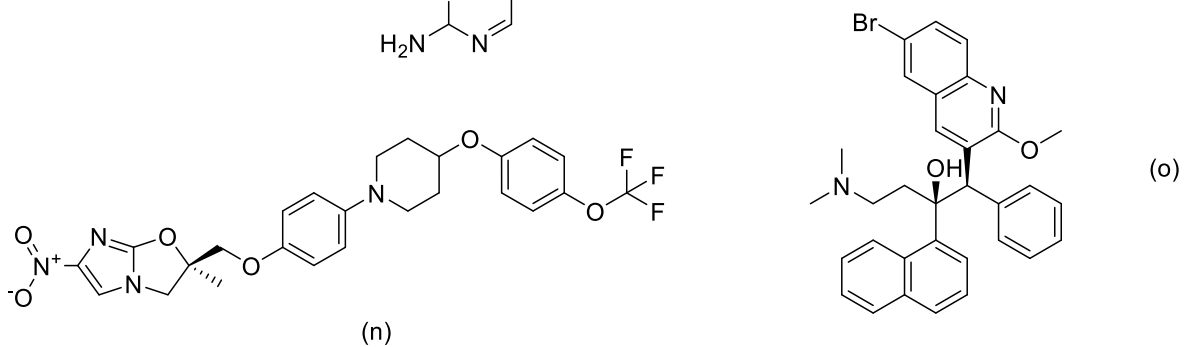
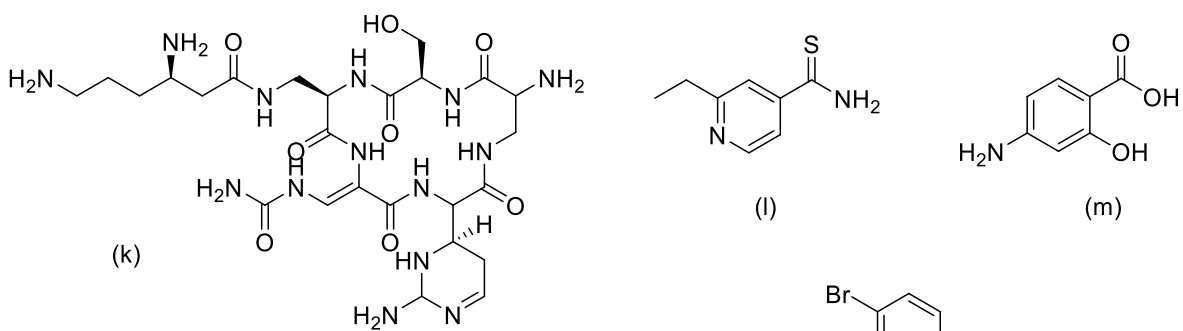
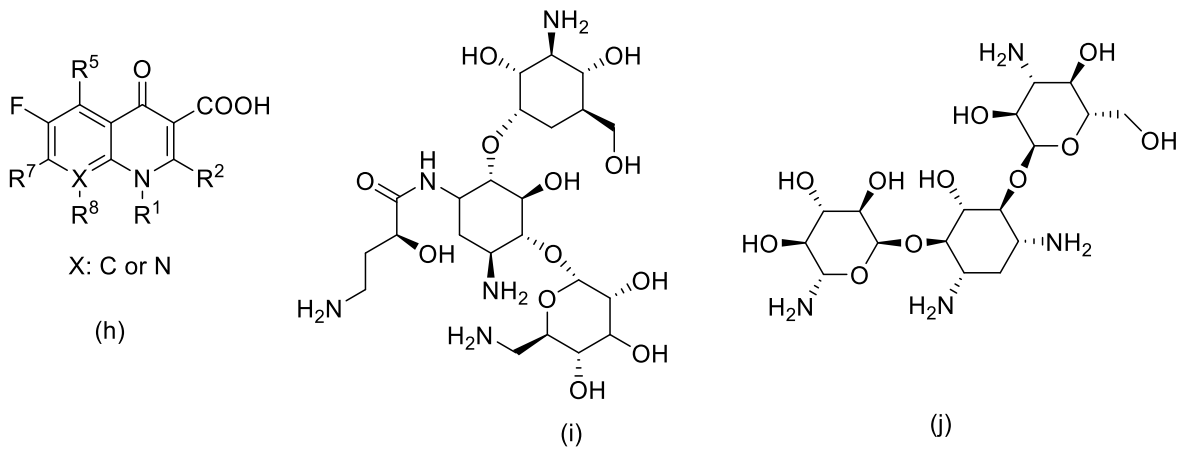
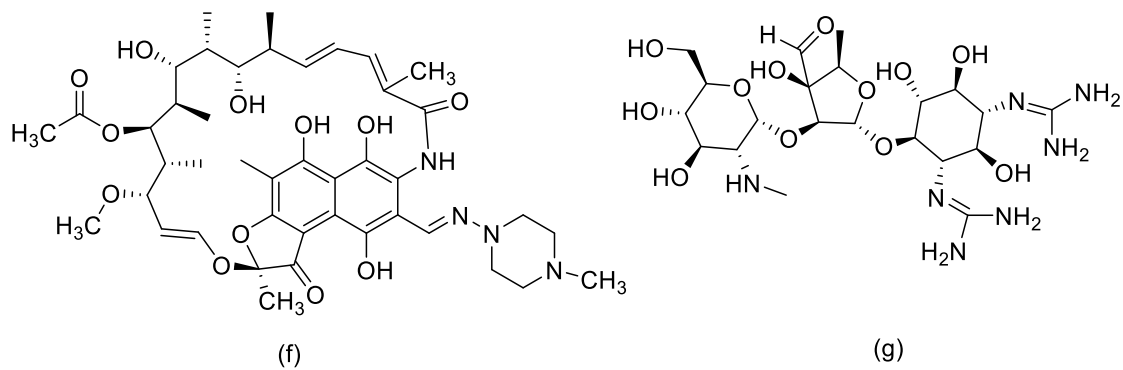
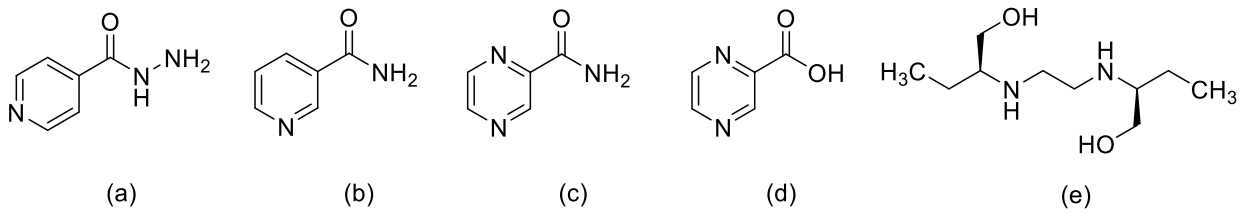


Figure 2. The chemical structure of (a) INH; (b) nicotinamide; (c) PZA; (d) POA; (e) ethambutol; (f) RFM; (g) streptomycin; (h) fluoroquinolones; (i) amikacin; (j) kanamycin; (k) capreomycin; (l) ethionamide; (m) *p*-aminosalicylic acid; (n) delamanid; and (o) bedaquiline.

Table 1: Examples of drug-drug interactions between anti-viral agents used in the management of HIV and the antitubercular RFM.

Anti-viral	Antitubercular	Interaction Outcome
Protease inhibitors	RFM	Increased frequency of nausea, vomiting, hepatitis [15]
Etravirine	RFM	Reduced plasma levels of etravirine [16]
Nevirapine	RFM	Increased risk of hepatotoxicity [17]

5.3. Pyrazinamide and Pyrazinamide Derivatives

Among antituberculars, PZA is a very interesting compound that recruited global efforts for further research. Previously, its mechanism of action was poorly understood, yet in the past few years new specific mechanisms of actions were proven by which PZA exerts its antimycobacterial effect. It was previously known that PZA acts as a prodrug that is transferred into its active form, pyrazinoic acid (POA, Figure 2) in the cytoplasm, resulting in lowering the intracellular pH and the subsequent disturbance of cell membrane function and eventually cell death. Yet, this traditional concept was proven to be false since PZA has to be present in concentrations exceeding its minimal inhibitory concentration (MIC) by 10-folds to exert such effect. The new mechanisms include the inhibition of quinolinic acid phosphoribosyl transferase (QAPRTase) that is involved in the synthesis of nicotinamide adenine dinucleotide (NAD),^[18] inhibition of aspartate decarboxylase that is responsible for the synthesis of vitamin B5 and thus coenzyme A (CoA),^[19] and inhibition of Fatty acid synthase I (FAS I) that is important for mycolic acid synthesis.^[20]

The new advancement in exploring PZA mechanism of action opened the window for further structural modifications to PZA/POA backbone to develop new derivatives with potential antimycobacterial activity. For example, many POA esters and amides have been prepared

as an attempt to overcome mycobacterial pyrazinamidase inactivation since such compounds require different enzymes to liberate the active form.^[21] Our research group is focused on investigating the antimicrobial activity in general and antimycobacterial activity in particular of several pyrazinamide derivatives. One major synthesis line is pyrazine carboxamides with various substituents on the pyrazine ring and the amidic nitrogen (Figure 3). Examples of prepared compounds by our research group include derivatives of 3-aminopyrazine-2-carboxamides,^[22] *N*-benzylpyrazine-2-carboxamide derivatives,^[23] 3-chloropyrazine-2-carboxamides,^[24] 5-chloro-*N*(3-nitrophenyl)pyrazine-2-carboxamides,^[25] *N*-phenylpyrazine-2-carboxamides,^[26-28] among others.

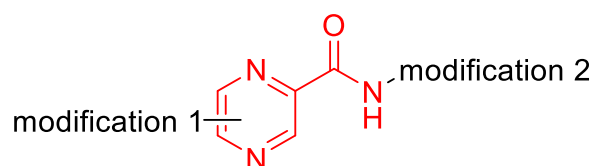


Figure 3: Structural modifications sites of pyrazine carboxamides prepared by our research group.

5.4. Title Compounds

In this diploma thesis, we report the synthesis of 16 quinoxaline-2-carboxylic acid derivatives; two of which (JZAS-13 and JZAS-15) had no CAS number when searched on SciFinder on February 20th, 2019. Only two compounds (JZAS-1 and JZAS-6) were previously mentioned in the literature as potential 5-HT₃ receptor antagonists for the management of depression and were not evaluated as antimicrobials. The current series of quinoxaline-2-carboxylic acid derivatives investigates the effect of quinoxaline core instead of pyrazine core “modification one” and the effect of various aromatic substituents at “modification 2” on biological activity as shown in general structure (A) in Figure 4. Several quinoxaline derivatives have been reported in the literature to have potent antimicrobial activities. For example olaquinox, quinoxin, and carbadox exerted antibacterial activity, while quinoxaline-2-carboxylate 1,4-dioxide and sugar conjugates of quinoxaline had potent

antimycobacterial activity.^[29, 30] The general structure (A) was then modified by adding methylene bridge to give general structure (B) in Figure 4.

Five out of the 16 prepared compounds belong to group A, *N*-phenylquinoxaline-2-carboxamides (JZAS-1, JZAS-3, JZAS-4, JZAS-2, SB-1), while the remaining 11 belong to group B, *N*-benzylquinoxaline-2-carboxamides (JZAS-6, JZAS-7, JZAS-8, JZAS-9, JZAS-13, JZAS-10, JZAS-11, JZAS-14, JZAS-14, JZAS-16, JZAS-17, JZAS-15).

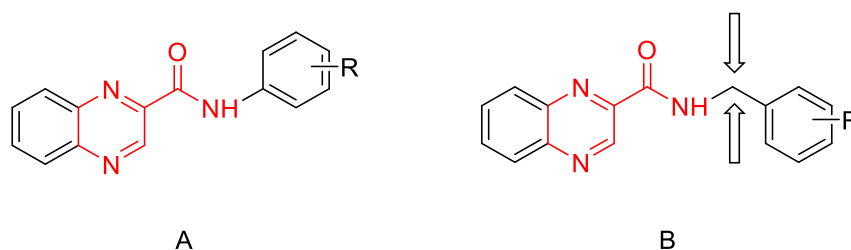


Figure 4: The general structures of title compounds.

All prepared compounds were evaluated for their anti-infective activities, including antimycobacterial against 6 mycobacterial strains (*Mtb* H37Ra, *Mtb* H37Rv, *M. kansasii*, *M. avium*, *M. smegmatis*, *M. aurum*) antibacterial activity against eight bacterial strains (*Staphylococcus aureus*, *Staphylococcus aureus* methicillin resistant, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Serratia marcescens*), and antifungal activity against eight fungal stems (*Candida albicans*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *Aspergillus flavus*, *Lichtheimia corymbifera*, *Trichophyton interdigitale*; *Aspergillus fumigatus*). Compounds were also evaluated for their *in vitro* cytotoxicity against HepG2 liver cancer cell line. The biological activities of compounds belonging to general structure B were compared to previously prepared, structurally related compounds from our group work (Figure 5), refer to section 7.1 for results and comparisons.^[31-33]

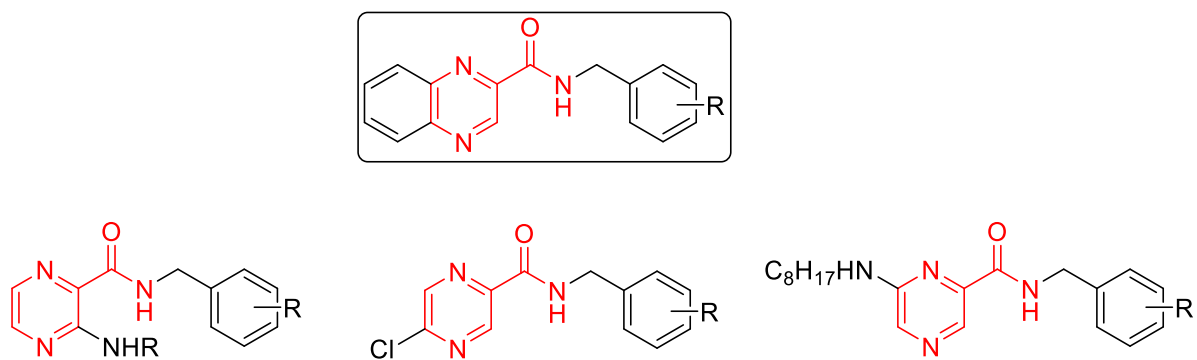


Figure 5: Demonstration of general structure B of title compounds (in rectangle) and other closely related general structures previously prepared by our research group.

6. EXPERIMENTAL

6.1. Instrumentation

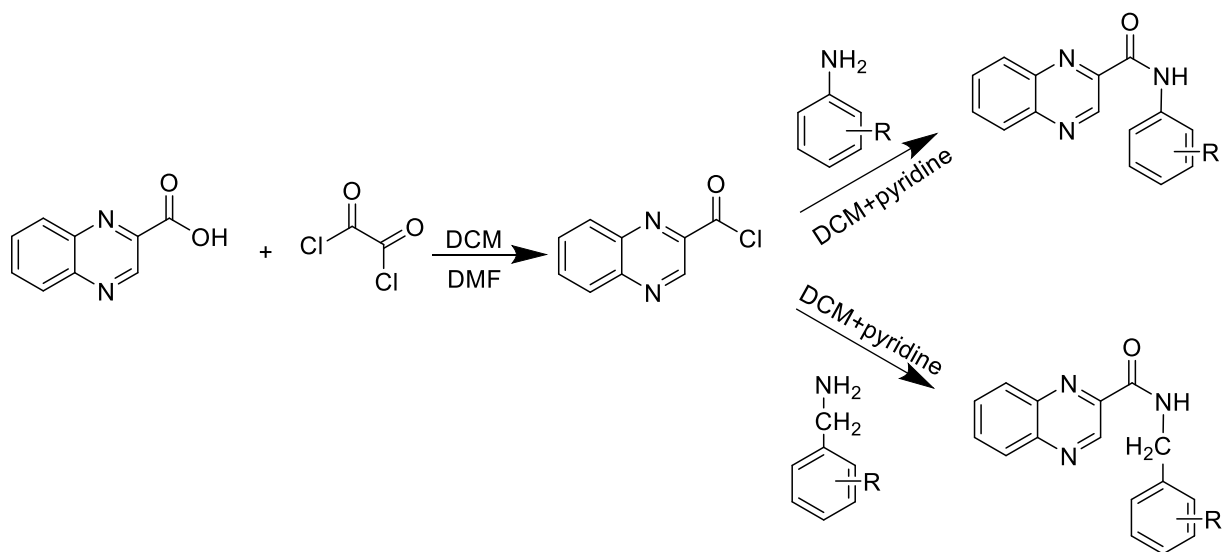
All chemical reactions were carried in normal laboratory glass equipment and at room temperature. The progress of the reaction was checked by Thin Layer Chromatography (TLC) (Alugram® Sil G/UV254, Machery-Nagel, Postfach, Germany) with UV detection using wavelength 254 nm. All obtained products were 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 mm, Merck, Darmstadt, Germany) was used as the stationary phase.

NMR spectra of prepared compounds were recorded on Varian VNMR S500 (499.87 MHz for ^1H 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). Melting points were assessed by SMP3 Stuart Scientific (Bibby Sterling Ltd., Staffordshire, UK) in open capillary. All chemical structures in this diploma were generated using the software CS ChemBioDraw Professional 17.0, and none was copied from the internet.

6.2. Derivatives of Quinoxaline-2-carboxylic Acid

6.2.1. Chemistry

The starting quinoxaline-2-carboxylic acid was activated with oxalyl chloride in anhydrous dichloromethane (DCM) as solvent in the presence of *N,N*-dimethyl formamide (DMF) and then reacted with 16 different aniline substituents in DCM and pyridine to yield the final compounds (Scheme 1).



Scheme 1: Synthesis of title compounds.

The final compounds were isolated mainly as white to yellow solids. Final compounds were fully characterized by their ¹H- and ¹³C-NMR spectra, IR spectra, melting point and elemental analysis. The obtained data were fully consistent with the proposed structures. In the IR spectra, the final compounds showed a signal at 1656–1720 cm⁻¹, attributed to the amidic carbonyl, a signal at 3303–3389 cm⁻¹ attributed to N-H bond of carbonyl, and for compounds belonging to group B, a characteristic signal for methylene bridge was observed at 2916–3098 cm⁻¹. In the ¹H-NMR spectra, the signal of the amidic hydrogen was dependent on the solvent used; it appeared as a broad singlet at 9.48–10.81 ppm in DMSO-d₆ and at 9.72–9.96 ppm in CDCl₃. The signal of the pyrazine hydrogen appeared at 9.76–8.28 ppm, independent of the solvent. In the ¹³C-NMR spectra, the signal of the amidic carbon appeared at 162.11–168.81 ppm. Representative NMR spectra of JZAS-1 and JZAS-10 are in section 6.3.

6.2.2. General Procedure

Quinoxaline-2-carboxylic acid (2 mmol, 348 mg) was dissolved in 15 mL of anhydrous DCM in a 50 mL Erlenmeyer flask with stirring. In a separate 10 mL Erlenmeyer flask, oxalyl chloride (2 mmol, 254 mg) was dissolved in 3 mL of anhydrous DCM, and then oxalyl chloride solution was added to the first Erlenmeyer flask. 1 drop DMF was then added to the

reaction mixture. The mixture was covered with parafilm and let stir at room temperature for 30 minutes (until no more effervescence by evolving gas – full activation of acid).

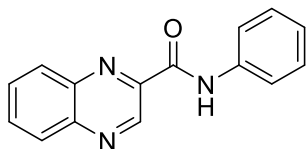
Meanwhile a solution of the selected aniline (for final compounds of group A) or benzylamine (for final compounds of group B) was prepared by dissolving 2 mmol of corresponding aniline or benzylamine in 20 mL of anhydrous DCM in a 50 mL Erlenmeyer flask with stirring. Pyridine (4.5 mmol, 356 mg) was then added to the solution and the mixture was covered with parafilm and put into ice-bath to cool.

After 15 minutes, the content of the first Erlenmeyer flask (acyl chloride) was slowly added (drop wise) to the aniline or benzylamine solution which is being stirred and cooled by ice. The ice-cooling bath was removed after 30 min and reaction is left overnight.

The reaction was stopped on the next day and pyridine was neutralized with 10% HCl. The organic layer (DCM) was washed with equal volume of water (40 mL). The added acid ensured that any unreacted anilines are ionized and will go to the water layer. The organic layer was washed two more times with water (2x 40 mL) and last time with brine (20 mL). The organic layer was then stirred with anhydrous Na_2SO_4 for 5 min and then Na_2SO_4 was filtered off using cotton. The obtained solution was evaporated under vacuum and adsorbed to silica gel to purify it with flash chromatography using gradient elution 0 to 100% EtOAc in hexane. Yields are expressed as percentages of theoretical yields and refer to the isolated products (chromatographically pure) after all purification steps.

6.2.3. Title Compounds

N-phenylquinoxaline-2-carboxamide



Code: JZAS-1

CAS#: 37648-63-8 ^[34]

Chemical formula: C₁₅H₁₁N₃O

Molecular weight: 249.27 g/mol

Yield: 50%

Appearance: White solid powder

m.p.: 182.2–183.5 °C

Rf (EtOAc /Hexane 1:1): 0.68

¹H-NMR (500 MHz, DMSO-*d*₆): δ 10.81 (s, 1H, amide), 9.54 (s, 1H, pyrazine), 8.34 – 8.27 (m, 1H, aromatic), 8.26 – 8.18 (m, 1H, aromatic), 8.06 – 7.99 (m, 2H, aromatic), 7.97 – 7.90 (m, 2H, aromatic), 7.45 – 7.37 (m, 2H, aromatic), 7.21 – 7.11 (m, 1H, aromatic)

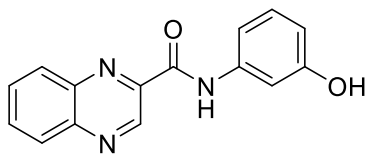
(in this and the following interpretations, by ‘pyrazine’ we mean the hydrogen atom present at C3 of the quinoxaline core)

¹³C-NMR (126 MHz, DMSO-*d*₆): δ 162.17, 144.90, 144.13, 143.12, 139.83, 138.34, 132.27, 132.02, 131.55, 129.74, 129.30, 128.94, 128.57, 124.50, 120.75

IR (ATR-Ge, cm⁻¹): 3361 (NH, CONH), 1676 (CO, CONH), 1596, 1571, 1532 (aromatic)

Elemental analysis: Calculated: C, 72.28%; H, 4.45%; N, 16.86%. Found: C, 71.85%; H, 4.33%; N, 16.66%

***N*-(3-hydroxyphenyl)quinoxaline-2-carboxamide**



Code: JZAS-3

CAS#: 1206970-59-3

Chemical formula: C₁₅H₁₁N₃O₂

Molecular weight: 265.27 g/mol

Yield: 80%

Appearance: yellow-orange solid powder

m.p.: 238.1–241.2 °C

R_f (EtOAc /Hexane 1:1): 0.44

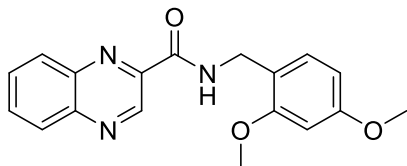
¹H-NMR (500 MHz, DMSO-*d*₆): δ 10.65 (s, 1H, amide), 9.55 – 9.49 (m, 2H, pyrazine, phenol), 8.33 – 8.26 (m, 2H, aromatic), 8.26 – 8.16 (m, 2H, aromatic), 7.51 (t, *J* = 2.2 Hz, 2H, aromatic), 7.33 – 7.28 (m, 2H, aromatic)

¹³C-NMR (126 MHz, DMSO-*d*₆): δ 162.32, 158.11, 145.26, 144.39, 143.37, 140.09, 139.60, 132.52, 131.80, 130.04, 129.89, 129.55, 111.94, 111.75, 108.04

IR (ATR-Ge, cm⁻¹): 3376 (NH, CONH), 1726 (CO, CONH), 1684, 1598, 1563 (aromatic)

Elemental analysis: Calculated: C, 67.92%; H, 4.18%; N, 15.84%. Found: C, 67.81%; H, 4.16%; N, 15.54%

***N*-(4-hydroxyphenyl)quinoxaline-2-carboxamide**



Code: JZAS-4

CAS#: 1912839-05-4

Chemical formula: C₁₅H₁₁N₃O₂

Molecular weight: 265.27 g/mol

Yield: 68%

Appearance: orange-red solid powder

m.p.: 224.7–226.1 °C

R_f (EtOAc /Hexane 1:1): 0.37

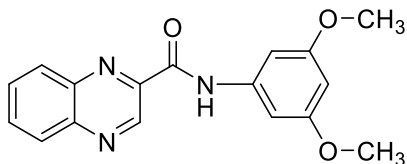
¹H-NMR (500 MHz, DMSO-*d*₆): δ 10.60 (s, 1H, amide), 9.52 (d, *J* = 0.8 Hz, 1H), 9.40 – 9.34 (m, 1H, pyrazine), 8.31 – 8.24 (m, 1H, aromatic), 8.26 – 8.17 (m, 1H, aromatic), 8.03 – 7.96 (m, 2H, aromatic), 7.74 – 7.67 (m, 2H, aromatic), 6.83 – 6.76 (m, 2H, aromatic)

¹³C-NMR (126 MHz, DMSO-*d*₆): δ 162.04, 157.83, 144.98, 144.11, 143.10, 139.82, 139.33, 132.24, 131.52, 129.76, 129.62, 129.28, 111.67, 111.47, 107.76.

IR (ATR-Ge, cm⁻¹): 3366 (NH, CONH), 1674 (CO, CONH), 1614, 1538, 1516 (aromatic)

Elemental analysis: Calculated: C, 67.92%; H, 4.18%; N, 15.84%. Found: C, 67.67%; H, 4.01%; N, 15.34%

***N*-(3,5-dimethoxyphenyl)quinoxaline-2-carboxamide**



Code: JZAS-2

CAS# 689265-59-6

Chemical formula: C₁₇H₁₅N₃O₃

Molecular weight: 309.33 g/mol

Yield: 70%

Appearance: yellow fluffy solid

m.p.: 184.2–186.1 °C

R_f (EtOAc /Hexane 1:1): 0.56

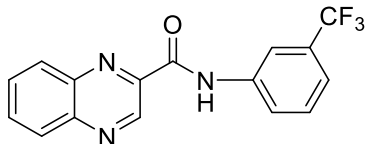
¹H-NMR (500 MHz, DMSO-*d*₆): δ 10.72 (s, 1H, amide), 9.53 (s, 1H, pyrazine), 8.33 – 8.26 (m, 1H, aromatic), 8.24 – 8.18 (m, 1H, aromatic), 8.06 – 7.97 (m, 2H, aromatic), 7.26 (d, *J* = 2.2 Hz, 2H, aromatic), 6.31 (t, *J* = 2.2 Hz, 1H, aromatic), 3.76 (s, 6H, CH₃)

¹³C-NMR (126 MHz, DMSO-*d*₆): δ 162.14, 161.58, 160.65, 144.71, 144.09, 143.13, 141.47, 140.00, 139.77, 132.30, 131.56, 129.71, 129.30, 128.70, 98.89, 96.59, 55.38

IR (ATR-Ge, cm⁻¹): 3339 (NH, CONH), 1678 (CO, CONH), 1617, 1599, 1573 (aromatic)

Elemental analysis: Calculated: C, 66.01%; H, 4.89%; N, 13.58%. Found: C, 65.92%; H, 4.88%; N, 13.52%

***N*-(3-(trifluoromethyl)phenyl)quinoxaline-2-carboxamide**



Code: SB-1

CAS#: 689265-58-5

Chemical formula: C₁₆H₁₀F₃N₃O

Molecular weight: 317.27 g/mol

Yield: 88%

Appearance: white solid powder

m.p.: 198.2–200.2 °C

Rf (EtOAc /Hexane 1:1): 0.60

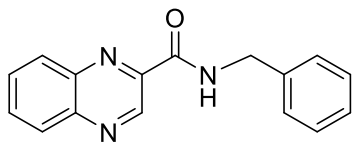
¹H-NMR (500 MHz, Chloroform-*d*): δ 9.96 (s, 1H, amide), 9.76 (s, 1H, pyrazine), 8.26 – 8.15 (m, 2H, aromatic), 8.13 (d, *J* = 2.0 Hz, 1H, aromatic), 8.09 – 8.03 (m, 1H, aromatic), 7.96 – 7.86 (m, 2H, aromatic), 7.55 (t, *J* = 7.9 Hz, 1H, aromatic), 7.48 – 7.43 (m, 1H, aromatic)

¹³C-NMR (126 MHz, Chloroform-*d*): δ 161.19, 144.17, 143.71, 142.73, 139.92, 137.81, 132.07, 131.60 (q, *J* = 32.5 Hz), 131.23, 129.75, 129.64, 129.53, 123.81 (q, *J* = 272.4 Hz), 122.82, 121.30 (q, *J* = 3.9 Hz), 116.55 (q, *J* = 4.0 Hz).

IR (ATR-Ge, cm⁻¹): 3347 (NH, CONH), 1687 (CO, CONH), 1603, 1574, 1539 (aromatic)

Elemental analysis: Calculated: C, 60.57%; H, 3.18%; N, 13.24%

***N*-benzylquinoxaline-2-carboxamide**



Code: JZAS-6

CAS#: 7066-32-2 ^[34]

Chemical Formula: C₁₆H₁₃N₃O

Molecular weight: 263.30 g/mol

Yield: 61%

Appearance: white crystals

m.p.: 202.2–204.8 °C

R_f (EtOAc/Hexane 1:1): 0.26

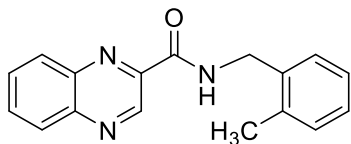
¹H-NMR (500 MHz, Chloroform-*d*): δ 9.73 (s, 1H, pyrazine), 8.33 (bs, 1H, amide), 8.20 (dd, *J* = 8.2, 1.7 Hz, 1H, aromatic), 8.08 (dd, *J* = 8.2, 1.7 Hz, 1H, aromatic), 7.91 – 7.79 (m, 2H, aromatic), 7.46 – 7.29 (m, 5H, aromatic), 4.76 (d, *J* = 6.1 Hz, 2H, NCH₂)

¹³C-NMR (126 MHz, DMSO-*d*₆): δ 163.20, 143.95, 143.34, 140.23, 137.82, 131.56, 130.79, 129.58, 129.51, 128.80, 127.93, 127.68, 43.57

IR (ATR-Ge, cm⁻¹): 3370 (NH, CONH), 2931(CH₂), 1670 (CO, CONH), 1585, 1524, 1516 (aromatic)

Elemental analysis: Calculated: C, 72.99%; H, 4.98%; N, 15.96%. Found: C, 72.62%; H, 4.88%; N, 15.83%

***N*-(2-methylbenzyl)quinoxaline-2-carboxamide**



Code: JZAS-7

CAS#: 930513-22-7

Chemical formula: C₁₇H₁₅N₃O

Molecular weight: 277.33 g/mol

Yield: 34%

Appearance: pale yellow powder

m.p.: 212.2–214.2 °C

R_f (EtOAc /Hexane 1:1): 0.60

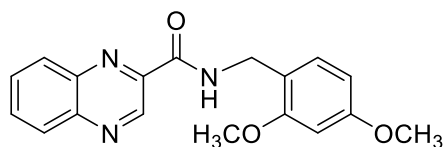
¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.49 (s, 1H, pyrazine), 9.43 (t, *J* = 6.2 Hz, 1H, amide), 8.23 – 8.15 (m, 2H, aromatic), 8.03 – 7.93 (m, 2H, aromatic), 7.34 – 7.28 (m, 1H, aromatic), 7.21 – 7.11 (m, 3H, aromatic), 4.56 (d, *J* = 6.2 Hz, 2H, NCH₂), 2.36 (s, 3H, CH₃)

¹³C-NMR (126 MHz, DMSO-*d*₆): 163.34, 144.56, 143.94, 143.14, 139.98, 136.91, 135.63, 132.02, 131.42, 130.07, 129.60, 129.26, 127.67, 127.03, 125.93, 40.58, 18.95

IR (ATR-Ge, cm⁻¹): 3314 (NH, CONH), 2916 (CH₂), 1656 (CO, CONH), 1627, 1591, 1576 (aromatic)

Elemental analysis: Calculated: C, 73.63%; H, 5.45%; N, 15.15%. Found: C, 73.38%; H, 5.23%; N, 14.95%

***N*-(2,4-dimethoxybenzyl)quinoxaline-2-carboxamide**



Code: JZAS-8

CAS#: 2183660-19-5

Chemical Formula: C₁₈H₁₇N₃O₃

Molecular weight: 323.35 g/mol

Yield: 24%

Appearance: beige solid powder

m.p.: 245.5–252.8 °C

R_f (EtOAc/Hexane 1:1): 0.30

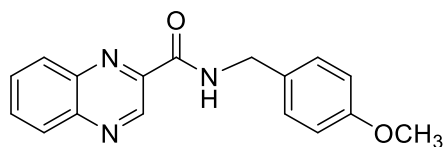
¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.48 (s, 1H, pyrazine), 9.15 (t, *J* = 6.3 Hz, 1H, amide), 8.23 – 8.15 (m, 2H, aromatic), 8.03 – 7.92 (m, 2H, aromatic), 7.16 (d, *J* = 8.3 Hz, 1H, aromatic), 6.58 (d, *J* = 2.4 Hz, 1H, aromatic), 6.47 (dd, *J* = 8.3, 2.4 Hz, 1H, aromatic), 4.49 (d, *J* = 6.2 Hz, 2H, NCH₂), 3.84 (s, 3H, OCH₃), 3.73 (s, 3H, OCH₃).

¹³C-NMR (126 MHz, DMSO-*d*₆): 163.13, 159.96, 157.87, 144.48, 143.85, 143.15, 139.93, 132.02, 131.42, 129.63, 129.24, 128.85, 118.59, 104.57, 98.47, 55.66, 55.36, 37.667

IR (ATR-Ge, cm⁻¹): 3303 (NH, CONH), 2909 (CH₂), 1662 (CO, CONH), 1607, 1571, 1537 (aromatic)

Elemental analysis: Calculated: C, 66.86%; H, 5.30%; N, 13.00%. Found: C, 66.63%; H, 5.28%; N, 12.92%

***N*-(4-methoxybenzyl)quinoxaline-2-carboxamide**



Code: JZAS-9

CAS#: 287945-56-6

Chemical formula: C₁₇H₁₅N₃O₂

Molecular weight: 293.33 g/mol

Yield: 58%

Appearance: light yellow solid powder

m.p.: 222.4–222.8 °C

R_f (EtOAc /Hexane 1:1): 0.37

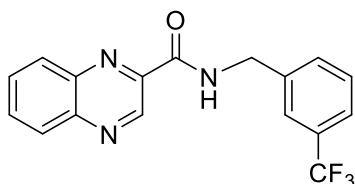
¹H-NMR (500 MHz, DMSO-*d*₆): 9.50 (t, *J* = 6.4 Hz, 1H, amide), 9.48 (s, 1H, pyrazine), 8.22 – 8.14 (m, 2H, aromatic), 8.03 – 7.92 (m, 2H, aromatic), 7.35 – 7.28 (m, 2H, aromatic), 6.94 – 6.83 (m, 2H, aromatic), 4.50 (d, *J* = 6.3 Hz, 2H, NCH₂), 3.72 (s, 3H, OCH₃)

¹³C-NMR (126 MHz, DMSO-*d*₆): δ 163.19, 161.35, 158.47, 144.60, 143.92, 143.12, 141.32, 139.96, 132.00, 131.42, 131.33, 129.56, 129.26, 129.12, 113.86, 55.21, 42.14

IR (ATR-Ge, cm⁻¹): 3303 (NH, CONH), 1662 (CH₂), 2909 (CO, CONH), 1614, 1585, 1571 (aromatic)

Elemental analysis: Calculated: C, 69.61%; H, 5.15%; N, 14.33%. Found: C, 69.29%; H, 5.14%; N, 13.98%

***N*-(3-(trifluoromethyl)benzyl)quinoxaline-2-carboxamide**



Code: JZAS-13

Chemical formula: C₁₇H₁₂F₃N₃O

Molecular weight: 331.30 g/mol

Yield: 47%

Appearance: pale yellow powder

m.p.: 211.3–213.4°C

R_f (EtOAc /Hexane 1:1): 0.47

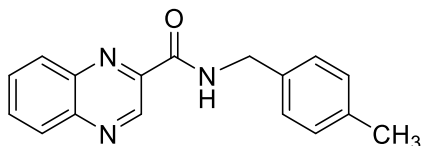
¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.74 (t, *J* = 6.4 Hz, 1H, amide), 9.48 (s, 1H, pyrazine), 8.23 – 8.15 (m, 2H, aromatic), 8.02 – 7.94 (m, 2H, aromatic), 7.77 – 7.73 (m, 1H, aromatic), 7.70 (d, *J* = 7.5 Hz, 1H, aromatic), 7.67 – 7.54 (m, 2H, aromatic), 4.66 (d, *J* = 6.3 Hz, 2H, NCH₂)

¹³C-NMR (126 MHz, DMSO-*d*₆): δ 163.60, 144.39, 143.95, 143.18, 140.89, 139.97, 132.07, 131.90, 131.46, 129.56, 129.28, 129.18 (*J* = 31.7 Hz), 124.42 (q, *J* = 272.4 Hz), 124.29 (q, *J* = 3.7 Hz), 123.82 (q, *J* = 3.5 Hz), 42.35 – signal of one aromatic C atom not distinguishable

IR (ATR-Ge, cm⁻¹): 3362 (NH, CONH), 2921 (CH₂), 1663 (CO, CONH), 1615, 1596, 1571 (aromatic)

Elemental analysis: Calculated: C, 61.63%; H, 3.56%; N, 12.68%.

***N*-(4-methylbenzyl)quinoxaline-2-carboxamide**



Code: JZAS-10

CAS#: 878922-59-9

Chemical formula: C₁₇H₁₅N₃O

Molecular weight: 277.33 g/mol

Yield: 56%

Appearance: white powder

m.p.: 205.4–207.2°C

R_f (EtOAc /Hexane 1:1): 0.50

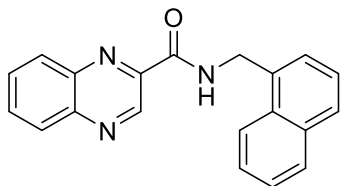
¹H-NMR (500 MHz, Chloroform-*d*): δ 9.72 (s, 1H, pyrazine), 8.28 (t, *J* = 5.9 Hz, 1H, amide), 8.19 (m, *J* = 8.4, 1.5 Hz, 1H, aromatic), 8.10 – 8.04 (m, 1H, aromatic), 7.84 (m, *J* = 20.6, 8.3, 6.9, 1.6 Hz, 2H, aromatic), 7.31 (d, *J* = 7.8 Hz, 2H, aromatic), 7.19 (d, *J* = 7.8 Hz, 2H, aromatic), 4.71 (d, *J* = 6.0 Hz, 2H, NCH₂), 2.36 (s, 3H, CH₃)

¹³C-NMR (126 MHz, Chloroform-*d*): δ 163.11, 161.35, 143.94, 143.90, 143.39, 141.32, 140.21, 137.42, 134.77, 131.50, 130.74, 129.56, 129.49, 129.44, 127.96, 43.35, 21.08

IR (ATR-Ge, cm⁻¹): 3348 (NH, CONH), 2926 (CH₂), 1671 (CO, CONH), 1574, 1533, 1494 (aromatic)

Elemental analysis: Calculated: C, 73.63%; H, 5.45%; N, 15.15%. Found: C, 73.01%; H, 5.35%; N, 14.83%

***N*-(naphthalen-1-ylmethyl)quinoxaline-2-carboxamide**



Code: JZAS-11

CAS#: 1444225-34-6

Chemical formula: C₂₀H₁₅N₃O

Molecular weight: 313.36 g/mol

Yield: 37%

Appearance: yellow-white powder

m.p.: 281.9–285.2 °C

R_f (EtOAc /Hexane 1:1): 0.48

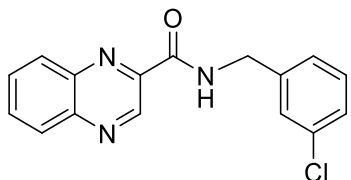
¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.60 (t, *J* = 6.2 Hz, 1H, amide), 9.52 (s, 1H, pyrazine), 8.28 (dd, *J* = 8.3, 1.2 Hz, 1H, aromatic), 8.23 – 8.15 (m, 2H, aromatic), 8.02 – 7.93 (m, 3H, aromatic), 7.86 (d, *J* = 8.2 Hz, 1H, aromatic), 7.62 – 7.51 (m, 3H, aromatic), 7.51 – 7.45 (m, 1H, aromatic), 5.06 (d, *J* = 6.1 Hz, 2H, NCH₂)

¹³C-NMR (126 MHz, DMSO-*d*₆): 163.38, 144.50, 143.97, 143.15, 139.96, 134.33, 133.46, 132.05, 131.43, 129.57, 129.57, 129.26, 128.69, 127.75, 126.46, 125.95, 125.79, 125.59, 123.65, 40.70

IR (ATR-Ge, cm⁻¹): 3369 (NH, CONH), 2921 (CH₂), 1677 (CO, CONH), 1625, 1600, 1574 (aromatic)

Elemental analysis: Calculated: C, 77.04%; H, 5.23%; N, 12.84%. Found: C, 77.16%; H, 5.28%; N, 12.85%

***N*-(3-chlorobenzyl)quinoxaline-2-carboxamide**



Code: JZAS-14

CAS#: 1794995-19-9

Chemical formula: C₁₆H₁₂ClN₃O

Molecular weight: 297.74 g/mol

Yield: 53%

Appearance: light yellow crystals

m.p.: 205.3–207.2°C

R_f (EtOAc /Hexane 1:1): 0.53

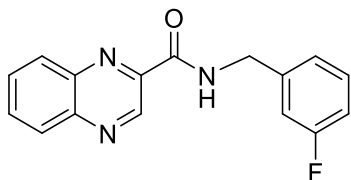
¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.81 (t, *J* = 6.4 Hz, 1H, amide), 9.48 (s, 1H, pyrazine), 8.23 – 8.09 (m, 4H, aromatic), 8.03 – 7.95 (m, 2H, aromatic), 7.86 (d, *J* = 7.7 Hz, 1H, aromatic), 7.67 – 7.59 (m, 1H, aromatic), 4.69 (d, *J* = 6.3 Hz, 2H, NCH₂)

¹³C-NMR (126 MHz, DMSO-*d*₆): 163.66, 147.97, 144.31, 143.94, 143.20, 141.76, 140.88, 139.97, 134.56, 132.12, 131.50, 130.02, 129.56, 129.30, 122.39, 122.10.

IR (ATR-Ge, cm⁻¹): 3389 (NH, CONH), 2938 (CH₂), 1673 (CO, CONH), 1599, 1574, 1521 (aromatic)

Elemental analysis: Calculated: C, 64.54%; H, 4.06%; N, 14.11%. Found: C, 64.37%; H, 4.06%; N, 13.95%

***N*-(3-fluorobenzyl)quinoxaline-2-carboxamide**



Code: JZAS-16

CAS#: 1387973-47-8

Chemical formula: C₁₆H₁₂FN₃O

Molecular weight: 281.29 g/mol

Yield: 51%

Appearance: white powder

m.p.: 201.3–205.1 °C

R_f (EtOAc /Hexane 1:1): 0.60

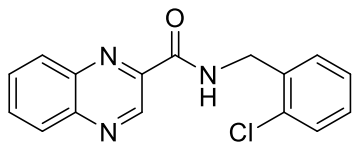
¹H-NMR (500 MHz, DMSO-*d*₆) δ 9.64 (t, *J* = 6.4 Hz, 1H, amide), 9.38 (s, 1H, pyrazine), 8.13 – 8.05 (m, 2H, aromatic), 8.02 – 7.91 (m, 2H, aromatic), 7.57 – 7.53 (m, 1H, aromatic), 7.50 (d, *J* = 7.5 Hz, 1H, aromatic), 7.47 – 7.44 (m, 2H, aromatic), 4.46 (d, *J* = 6.3 Hz, 2H, NCH₂)

¹³C-NMR (126 MHz, DMSO-*d*₆) δ 168.81, 162.11 (d), 156.80, 143.54, 141.49, 140.62, 131.59, 131.13, 130.78, 129.87, 128.64, 124.55, 122.26, 116.47, 40.59

IR (ATR-Ge, cm⁻¹): 3308 (NH, CONH), 2958 (CH₂), 1719 (CO, CONH), 1655, 1615, 1590 (aromatic)

Elemental analysis: Calculated: C, 68.32%; H, 4.30%; N, 14.94%.

***N*-(2-chlorobenzyl)quinoxaline-2-carboxamide**



Code: JZAS-17

CAS#: 878110-49-7

Chemical formula: C₁₆H₁₂ClN₃O

Molecular weight: 297.74 g/mol

Yield: 28%

Appearance: light yellow powder

m.p.: 204.4–206.7°C

R_f (EtOAc /Hexane 1:1): 0.82

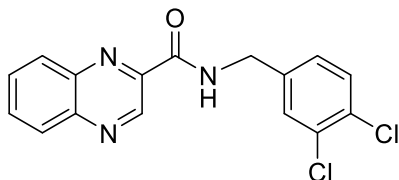
¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.61 (t, *J* = 6.2 Hz, 1H, amide), 9.49 (s, 1H, pyrazine), 8.26 – 8.16 (m, 2H, aromatic), 8.05 – 7.95 (m, 2H, aromatic), 7.51 – 7.38 (m, 2H, aromatic), 7.36 – 7.26 (m, 2H, aromatic), 4.65 (d, *J* = 6.2 Hz, 2H, CH₂).

¹³C-NMR (126 MHz, DMSO-*d*₆): not available

IR (ATR-Ge, cm⁻¹): 3378 (NH, CONH), 3098 (CH₂), 1715 (CO, CONH), 1663, 1581, 1523 (aromatic)

Elemental analysis: Calculated: C, 64.54%; H, 4.06%; N, 14.11%. Found: C, 64.17%; H, 4.02%; N, 13.95%

***N*-(3,4-dichlorobenzyl)quinoxaline-2-carboxamide**



Code: JZAS-15

Chemical Formula: C₁₆H₁₁Cl₂N₃O

Molecular weight: 332.18 g/mol

Yield: 49%

Appearance: yellow solid

m.p.: 203.5–205.5°C

R_f (EtOAc/Hexane 1:1): 0.41

¹H-NMR ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.70 (t, *J* = 6.4 Hz, 1H, amide), 9.48 (s, 1H, pyrazine), 8.23 – 8.16 (m, 2H, aromatic), 8.02 – 7.95 (m, 2H, aromatic), 7.64 (d, *J* = 2.1 Hz, 1H, aromatic), 7.58 (d, *J* = 8.3 Hz, 1H, aromatic), 7.38 (dd, *J* = 8.3, 2.1 Hz, 1H, aromatic), 4.56 (d, *J* = 6.3 Hz, 2H, CH₂).

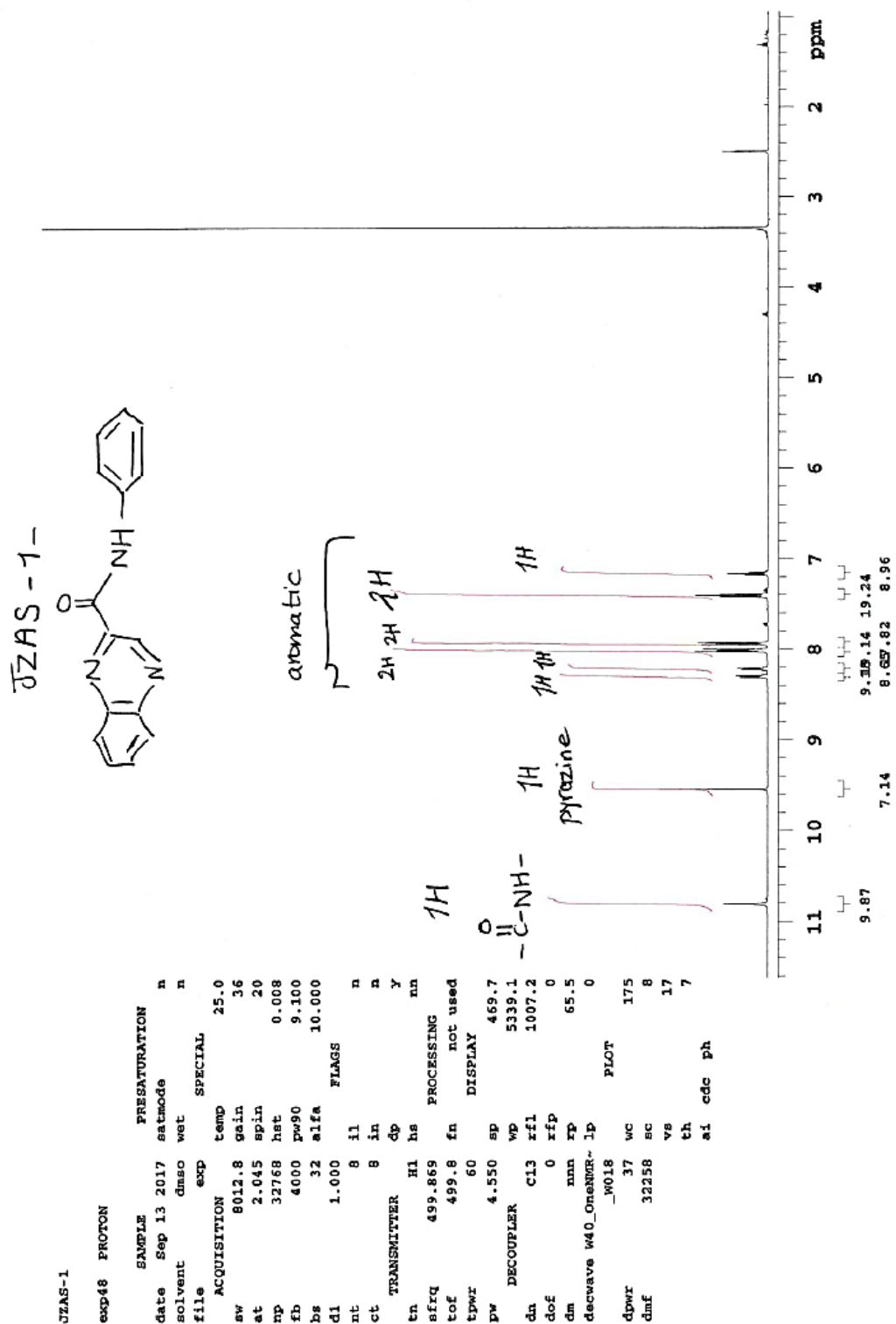
¹³C-NMR: 163.57, 144.33, 143.94, 143.17, 140.62, 139.95, 132.06, 131.45, 131.00, 130.62, 129.70, 129.58, 129.54, 129.27, 128.08, 41.72

IR (ATR-Ge, cm⁻¹): 3361 (NH, CONH), 2922 (CH₂), 1668 (CO, CONH), 1629, 1589, 1571 (aromatic)

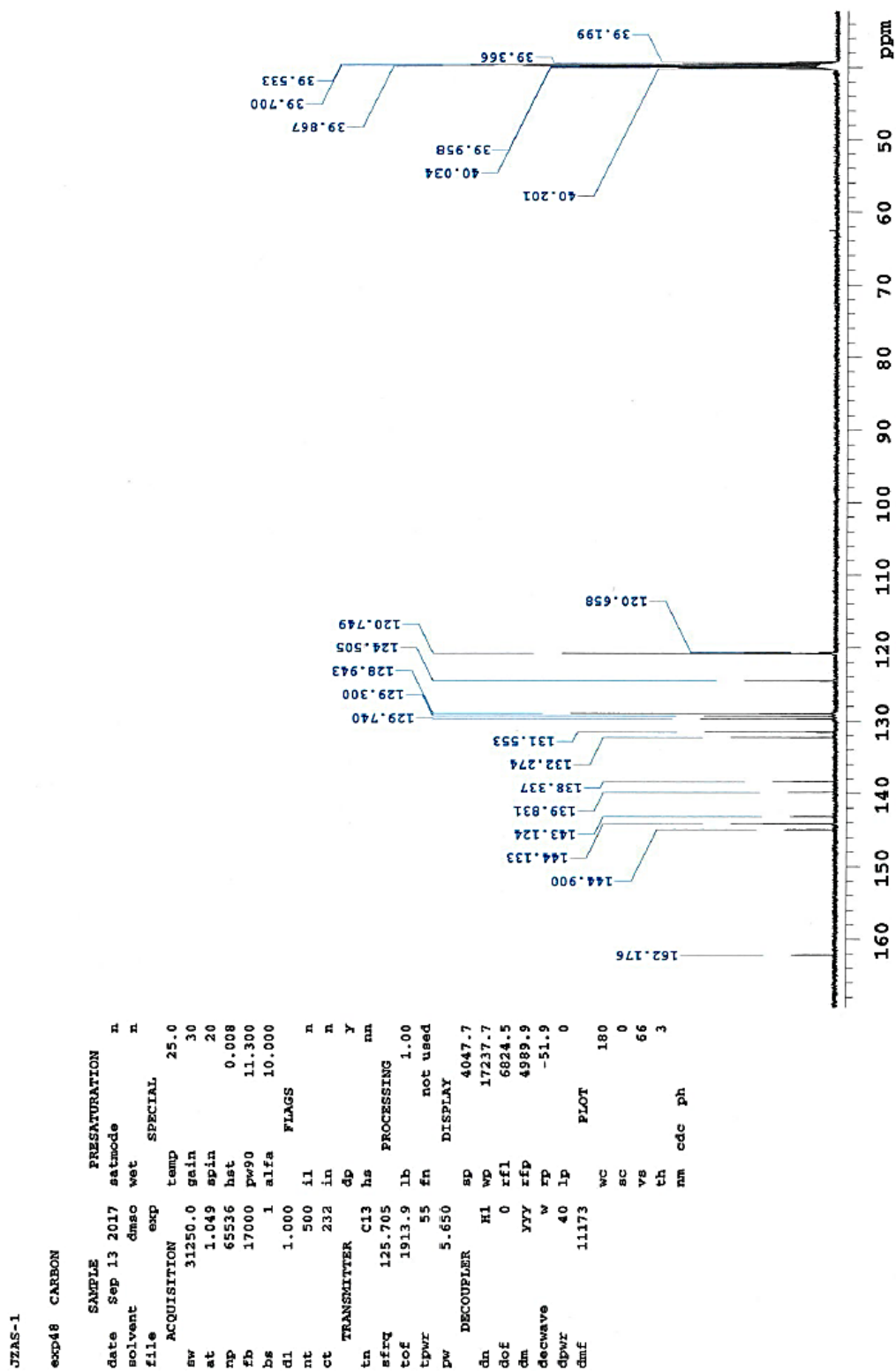
Elemental analysis: Calculated: C, 57.85%; H, 3.34%; N, 12.65%. Found: C, 57.60%; H, 3.31%; N, 12.45%

6.3. Selected Spectra of Some Prepared Compounds

6.3.1. ¹H-NMR Spectra of Compound JZAS-1

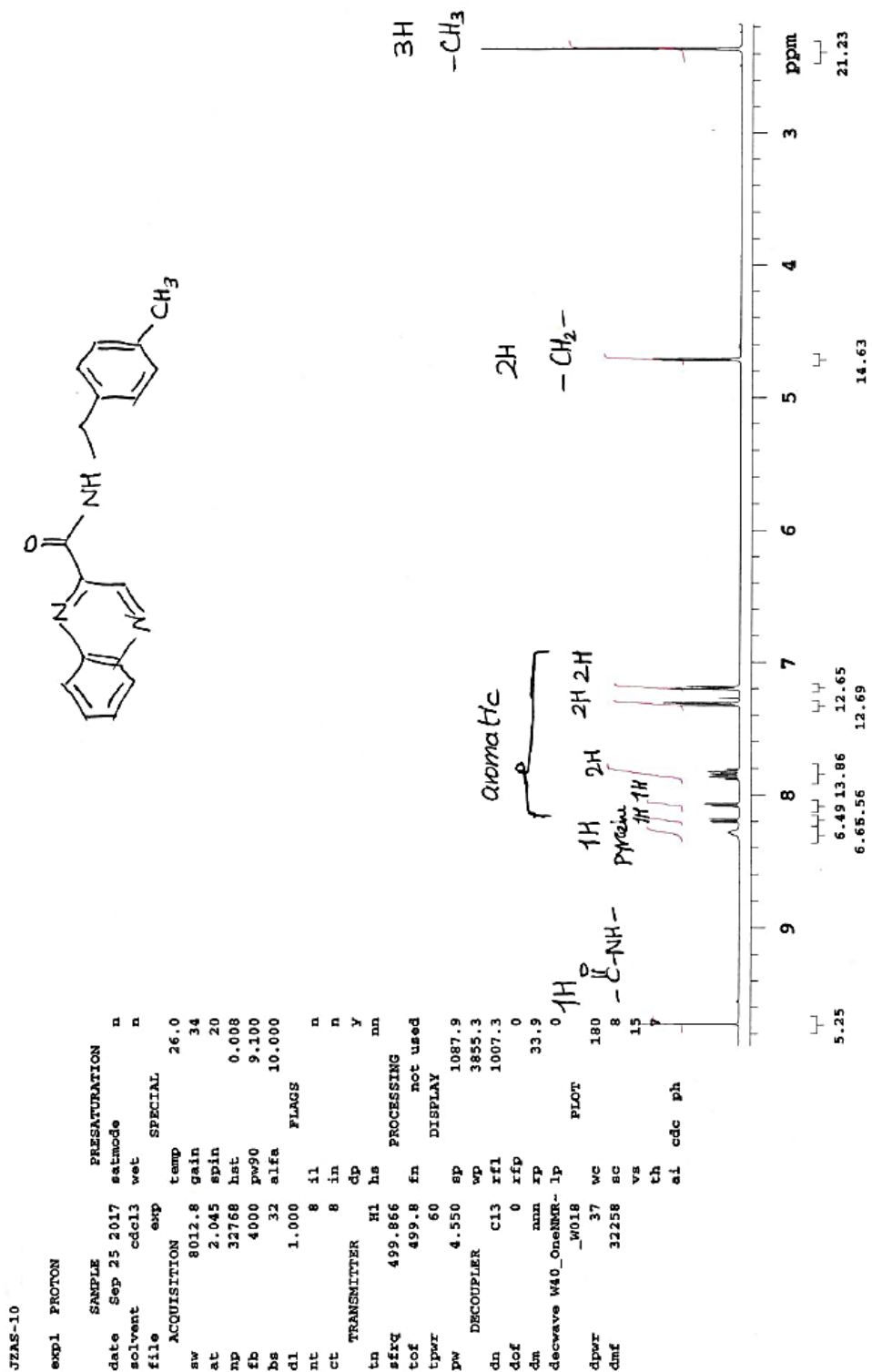
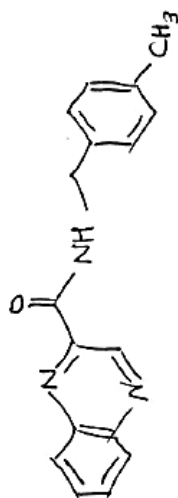


6.3.2. ¹³C-NMR Spectra of Compound JZAS-1

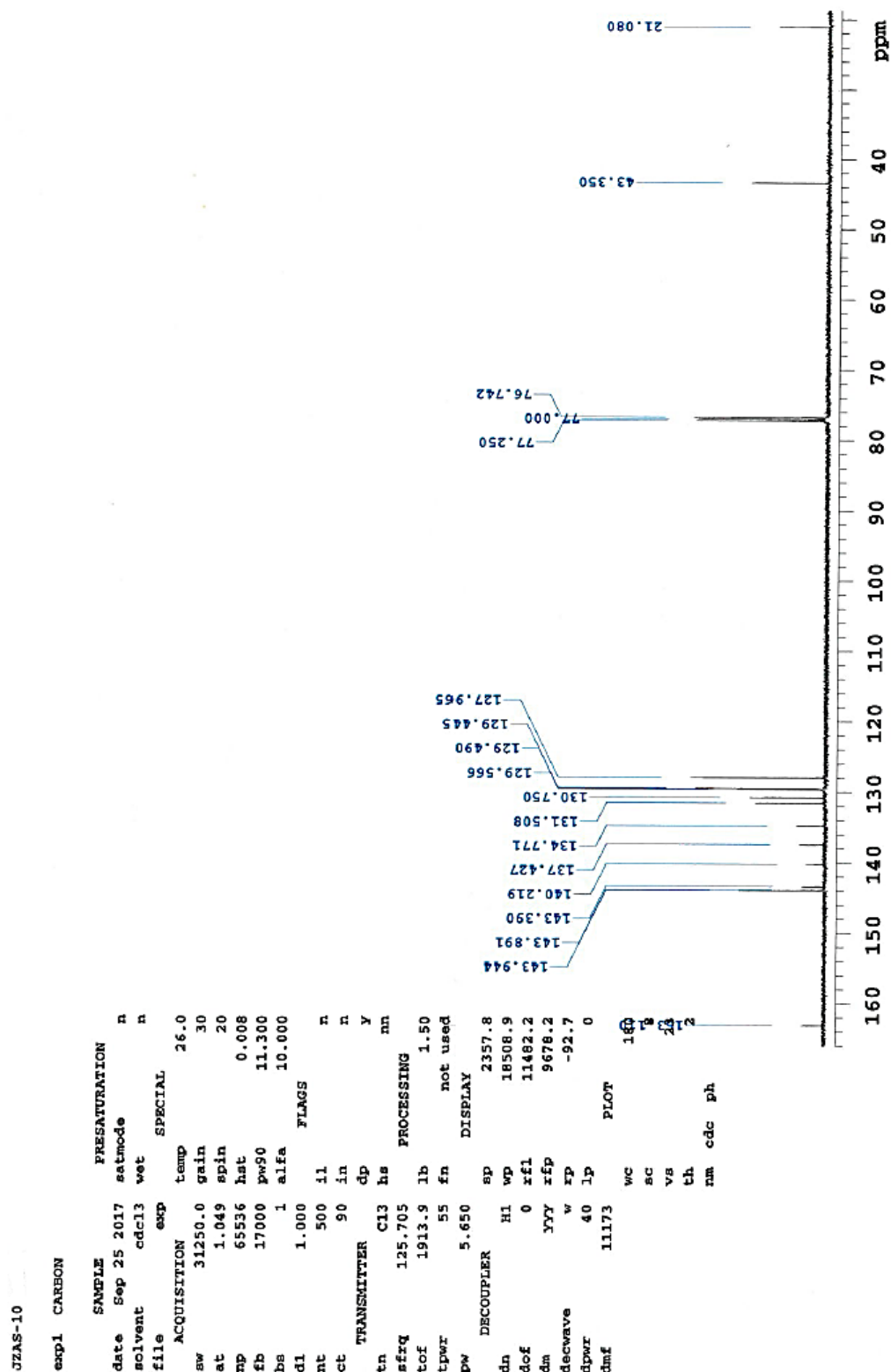


6.3.3. ¹H-NMR Spectra of Compound JZAS-10

JZAS-10



6.3.4. ¹³C-NMR Spectra of Compound JZAS-10



6.4. Biological Assays

6.4.1. *In Vitro* Activity Evaluation Against *Mycobacterium tuberculosis* H37Rv, *Mycobacterium kansasii*, and *Mycobacterium avium*

A 96-well plate microdilution broth method was performed. Tested strains *Mtb* H37Rv CNCTC My 331/88 (ATCC 27294), *M. kansasii* CNCTC My 235/80 (ATCC 12478) and *M. avium ssp. avium* CNCTC My 80/72 (ATCC 15769) were obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health (Prague, Czech Republic). Middlebrook 7H9 broth of declared pH = 6.6 (Sigma-Aldrich) enriched with 0.4% of glycerol (Sigma-Aldrich) and 10% of OADC growth supplement (oleic acid, albumin, dextrose, catalase; Himedia, Mumbai, India) was used for cultivation. Tested compounds were dissolved and diluted in DMSO and mixed with broth (25 μ L of DMSO solution in 4.475 mL of broth) and placed (100 μ L) into microplate wells. Mycobacterial inocula were suspended in isotonic saline solution and the density was adjusted to 0.5–1.0 according to McFarland scale. These suspensions were diluted by 10^{-1} and used to inoculate the testing wells, adding 100 μ L of mycobacterial suspension per well. Final concentrations of tested compounds in wells were 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 μ g/mL. INH was used as positive control (inhibition of growth). Negative control (visible growth) consisted of broth plus mycobacterial suspension plus DMSO (purity of broth). A total of 30 μ L of Alamar Blue working solution (1:1 mixture of 0.01% resazurin sodium salt (aq. sol.) and 10% Tween 80) was added after five days of incubation. Results were then determined after 24 h of incubation. The MIC (in μ g/mL) was determined as the lowest concentration that prevented the blue to pink color change. MIC values of INH were 6.25–12.5 μ g/mL against *M. avium*, 3.13–12.5 μ g/mL against *M. kansasii*, and 0.1–0.2 μ g/mL against *Mtb* H37Rv. All experiments were conducted in duplicates.

6.4.2. *In Vitro* Activity Evaluation Against *Mycobacterium smegmatis*, *Mycobacterium aurum*, and *Mycobacterium tuberculosis* H37Ra

Antimycobacterial assay was performed with fast growing *M. smegmatis* DSM 43465 (ATCC 607), *M. aurum* DSM 43999 (ATCC 23366) from German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and with avirulent strain of *Mtb* H37Ra ITM-M006710 (ATCC 9431) from Belgian Co-ordinated Collections of Micro-

organisms. The technique used for activity determination was microdilution broth panel method using 96-well microtitration plates. Culturing medium was Middlebrook 7H9 broth (Sigma-Aldrich, Steinheim, Germany) enriched with 0.4% of glycerol (Sigma-Aldrich, Steinheim, Germany) and 10% of Middlebrook OADC growth supplement (Himedia, Mumbai, India). Mycobacterial strains were cultured on Middlebrook 7H9 agar and suspensions were prepared in Middlebrook 7H9 broth. Final density was adjusted to value 1.0 according to McFarland scale and diluted in ratio 1:20 (for fast growing mycobacteria) or 1:10 (for *Mtb*) with broth. Tested compounds were dissolved in DMSO (Sigma-Aldrich, Steinheim, Germany) then MB broth was added to obtain concentration 2000 µg/mL. Standards used for activity determination were isoniazid (INH), rifampicin (RIF) and ciprofloxacin (CPX) (Sigma-Aldrich, Steinheim, Germany). 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 and 3.91 µg/mL. Isoniazid was diluted in range 5003.91 µg/mL for screening against fast growing mycobacteria and in range 10.0078 µg/mL for screening against *Mtb*. Rifampicin final concentrations ranged from 50 to 0.39 µg/mL for fast growing mycobacteria and from 1 to 0.0078 µg/mL for *Mtb*. Ciprofloxacin was used for screening antimycobacterial activity with the final concentrations 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*, *M. aurum* nor *Mtb*. Positive (broth, DMSO, bacteria) and negative (broth, DMSO) controls were included. Plates were sealed with polyester adhesive film and incubated in dark at 37°C without agitation. The addition of 0.01% solution of resazurin sodium salt followed after 48 hours of incubation for *M. smegmatis*, after 72 hours of incubation for *M. aurum* and after 120 hours of incubation for *Mtb*, respectively. Stain was prepared by dissolving resazurin sodium salt (Sigma-Aldrich, Steinheim, Germany) in deionised water to get 0.02% solution. Then 10% aqueous solution of Tween 80 (Sigma-Aldrich, Steinheim, Germany) was prepared. Both liquids were mixed up making use of the same volumes and filtered through syringe membrane filter. Microtitration panels were then incubated for further 2.5 hours for determination of activity against *M. smegmatis*, 4 hours for *M. aurum* and 24 hours for *Mtb*, respectively. 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 – not active 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*, and 1.95-3.91 µg/mL for INH, 0.39-0.78 µg/mL for RIF and 0.0078-0.0156 µg/mL for CPX against *M. aurum*, and 0.125-0.25 µg/mL for INH, 0.0039-0.0078 µg/mL for RIF and 0.125-0.25 for CPX against *Mtb*, respectively. All experiments were conducted in duplicate.

6.4.3. *In Vitro* Antibacterial Activity Evaluation

Microdilution broth method.^[35] Tested strains from the Czech Collection of Microorganisms (CCM, Brno, Czech Republic) - *Staphylococcus aureus* CCM 4223 (ATCC 29213), *Staphylococcus aureus* methicillin resistant CCM 4750 (ATCC 43300), *Enterococcus faecalis* CCM 4224 (ATCC 29212), *Escherichia coli* CCM 3954 (ATCC 25922), *Pseudomonas aeruginosa* CCM 3955 (ATCC 27853). Clinical isolates from the Department of Clinical Microbiology, University Hospital in Hradec Králové, 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 °C and maintained on the same medium at 4 °C. 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. Positive controls consisted of test microbe solely, while negative controls consisted of cultivation medium and DMSO. The final concentration of DMSO in the testing medium did not exceed 1% (v/v) of the total solution composition. MIC was determined after 24 and 48 h of static incubation at 35 °C by visual inspection or using Alamar Blue dye. The standards were gentamicin and ciprofloxacin. The standards were gentamicin [MIC against *Staphylococcus aureus* 1 µg/mL (48 h); *Staphylococcus aureus* methicillin resistant 16–32 µg/mL (48 h); *Enterococcus faecalis* 8 µg/mL (48 h); *Escherichia coli* 1–2 µg/mL (48 h); *Pseudomonas aeruginosa* 0.5 µg/mL (48 h); *Staphylococcus epidermidis* >8 µg/mL (48 h); *Klebsiella pneumoniae* >8 µg/mL (48 h); *Serratia marcescens* 2 µg/mL (48 h)] and ciprofloxacin [MIC against *Staphylococcus aureus* 0.128–0.256 µg/mL (48 h); *Staphylococcus aureus* methicillin resistant 0.128 µg/mL (48 h); *Enterococcus faecalis* 0.512 µg/mL (48 h); *Escherichia coli* 0.008 µg/mL (48 h); *Pseudomonas aeruginosa* 0.128 µg/mL (48 h);

Staphylococcus epidermidis >1.024 µg/mL (48 h); *Klebsiella pneumonia* >1.024 µg/mL (48 h); *Serratia marcescens* 0.256 µg/mL (48 h)]. All 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.

6.4.4. *In Vitro* Antifungal Activity Evaluation

Microdilution broth method.^[36] Tested 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, *Lichtheimia corymbifera* CCM 8077 and *Trichophyton interdigitale* CCM 8377 (ATCC 9533); or from the American Type Collection Cultures (ATCC, Manassas, 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 with MOPS (3-morpholinopropane-1-sulfonic acid). The final concentration of DMSO in the testing medium did not exceed 1% (v/v) of the total solution composition. Static incubation was performed in the dark and in humid atmosphere, at 35 °C, for 24 and 48 h (72 and 120 h for *Trichophyton interdigitale* respectively). Positive controls consisted of test microbe solely, while negative controls consisted of cultivation medium and DMSO. MIC was inspected visually or making use of Alamar Blue indication. The standards were amphotericin B [MIC against *Candida albicans* 0.5 µg/mL (48 h); *C. krusei* 1 µg/mL (48 h); *C. parapsilosis* 0.5 µg/mL (48 h); *C. tropicalis* 1 µg/mL (48 h); *Aspergillus flavus* 8 µg/mL (48 h); *Lichtheimia corymbifera* 0.5 µg/mL (48 h); *Trichophyton interdigitale* 2 µg/mL (72 h); *Aspergillus fumigatus* 1 µg/mL (48 h)] and voriconazole [MIC against *Candida albicans* >16 µg/mL (48 h); *C. krusei* 0.5 µg/mL (48 h); *C. parapsilosis* 8 µg/mL (48 h); *C. tropicalis* >16 µg/mL (48 h); *Aspergillus flavus* >16 µg/mL (48 h); *Lichtheimia corymbifera* >16 µg/mL (48 h); *Trichophyton interdigitale* >16 µg/mL (72 h); *Aspergillus fumigatus* 1 µg/mL (48 h)]. All 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.

6.4.5. Cytotoxicity

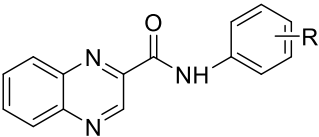
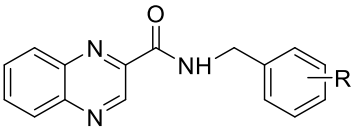
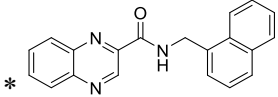
The human hepatocellular liver carcinoma cell line HepG2 purchased from Health Protection Agency Culture Collections (ECACC, Salisbury, UK) was cultured in MEM (Minimum Essentials Eagle Medium) (SIGMA ALDRICH, St. Louis, USA) supplemented with 10% fetal bovine serum (PAA), 1% L-Glutamine solution (SIGMA ALDRICH, St. Louis, USA) and non-essential amino acid solution (SIGMA ALDRICH, St. Louis, USA) in a humidified atmosphere containing 5% CO₂ at 37°C. For subculturing, the cells were harvested after trypsin/EDTA (SIGMA ALDRICH, St. Louis, USA) treatment at 37°C. To evaluate cytotoxicity, the cells treated with the tested substances were used as experimental groups whereas untreated HepG2 cells served as controls. The cells were seeded in density 10 000 cells per well in a 96-well plate. Next day the cells were treated with each of the tested substances dissolved in DMSO. The tested substances were prepared at different incubation concentrations in triplicates according to their solubility. Simultaneously, the controls representing 100% cell viability, 0% cell viability (the cells treated with 10% DMSO), no cell control and vehiculum controls were also prepared in triplicates. After 24 h incubation in a humidified atmosphere containing 5% CO₂ at 37%, the reagent from the kit CellTiter 96 AQueous One Solution Cell Proliferation Assay (CellTiter 96; PROMEGA, Fitchburg, USA) was added. After 2h incubation at 37% absorbance of samples was recorded at 490 nm (TECAN, Infinita M200, Austria). A standard toxicological parameter IC₅₀ was calculated by nonlinear regression from a semilogarithmic plot of incubation concentration versus percentage of absorbance relative to untreated controls using GraphPad Prism 8 software. Results of the experiments are presented as inhibitory concentration which reduces viability of the cell population to 50% from the maximal viability (IC₅₀). Table 4 and Figure 8 show a comparison of the found cytotoxic effect of the tested compounds in HepG2 cell line using the methods CellTiter 96.

7. RESULTS AND DISCUSSION

7.1. In Vitro Activity Evaluation Against *Mycobacterium tuberculosis* H37Ra, *Mycobacterium tuberculosis* H37Rv, *Mycobacterium kansasii*, and *Mycobacterium avium*

All prepared compounds were evaluated for antimycobacterial activity against *Mtb* H37Ra, *Mtb* H37Rv, *M. kansasii*, and *M. avium*. Obtained biological activities are summarized in Table 2.

Table 2: Prepared compounds, calculated partition coefficients log *P*, and antimycobacterial activity against *Mtb* H37Ra, *Mtb* H37Rv, *M. kansasii*, and *M. avium* in µg/mL.

						
Cmpd.	R	Log <i>P</i>	<i>Mtb</i> H37Ra	<i>Mtb</i> H37Rv	<i>M. kansasii</i>	<i>M. avium</i>
JZAS-1	H	2.43	≥500 (≥ 2006 µM)	>100	>100	>100
JZAS-3	3-OH	2.04	15.625 (59 µM)	>100	>100	>100
JZAS-4	4-OH	2.04	62.5 (236 µM)	>100	>100	>100
JZAS-2	3,5-diOCH ₃	2.18	62.5 (202 µM)	>100	>100	>100
SB-1	3-CF ₃	3.35	≥500 (≥1576 µM)	>100	>100	>100
						
Cmpd.	R		<i>Mtb</i> H37Ra	<i>Mtb</i> H37Rv	<i>M. kansasii</i>	<i>M. avium</i>
JZAS-6	H	2.5	15.625 (59 µM)	>100	>100	>100
JZAS-7	2-CH ₃	2.99	7.81 (28 µM)	>100	>100	>100
JZAS-8	2,5-diOCH ₃	2.25	3.91 (12.1 µM)	>50	>50	>50
JZAS-9	4-OCH ₃	2.38	3.91 (13 µM)	25	50	50
JZAS-13	3-CF ₃	3.42	3.91 (11.8 µM)	>100	>100	>100
JZAS-10	4-CH ₃	2.99	7.81 (28.2 µM)	50	>100	>100
JZAS-11	*	3.5	≥250 (797.8 ≥ µM)	>100	>100	>100
JZAS-14	3-Cl	3.06	7.81 (26.2 µM)	25	50	25
JZAS-16	3-F	2.66	7.81 (27.8 µM)	>100	>100	>100
JZAS-17	2-Cl	3.06	15.625 (52.5 µM)	>100	>100	>100
JZAS-15	3,4-diCl	3.62	7.81 (23.5 µM)			
						

Based on obtained antimycobacterial activities against *Mtb* H37Ra, we find that most compounds exerted moderate to high activity. The most active compounds with MIC = 3.91 $\mu\text{g/mL}$ were JZAS-13 (11.8 μM), JZAS-8 (12.1 μM), and JZAS-9 (13 μM), all of which belong to the general structure B. In general, compounds belonging to the structural type B are more active. Two (SB-1 and JZAS-1) out of three non-active compounds (SB-1, JZAS-1, and JZAS-11) belong to the general structure A group. If we compare compounds belonging to the two groups bearing the same substituent (R), for example JZAS-1 vs. JZAS-6 (R = H) and SB-1 vs. JZAS-13 (R = 3-CF₃), we find that homologues belonging to group B overrule their corresponding structures of type A. These findings highlight the positive effect of introducing the methylene bridge on biological activity. This extra methylene added flexibility to the molecule and might have created an opportunity to form more hydrophobic interactions with target and thus improving the biological activity and or may have elongated the molecule in a way that permits better interacting of adjacent atoms with the target. Regarding the nature of the substituent (R), we can see that bulky substituents (3-CF₃, 4-OCH₃, 2,5-diOCH₃) favour antimycobacterial activity. Yet, JZAS-11 with naphthalene was inactive and this can be attributed to the additional benzene core that made the molecule perhaps too bulky for good receptor interaction. When we compare the effect of lipophilicity of the substituent (R) on biological activity, for example the more hydrophilic JZAS-3 (R = 3-OH, log *P* = 2.04) vs. the more lipophilic SB-1 (R = 3-CF₃, log *P* = 3.35) both of which are from structural group A, we can observe that JZAS-3 was active (MIC = 15.625 $\mu\text{g/mL}$) while SB-1 was inactive (MIC = ≥ 500 $\mu\text{g/mL}$). This suggests that more hydrophilic substituents (R) might contribute to better biological activity. Yet, in compounds belonging to general structural type B, there is no compounds prepared bearing a strong hydrophilic substituent (R). Since one the most active compound JZAS-13 (MIC = 3.91 $\mu\text{g/mL}$, 11.8 μM) had lipophilic substituent (R = 3-CF₃), it would be wise to prepare a new compound with a more hydrophilic substituent in the same position, for example R = 3-OH or 3-NH₂, and see if this observation can be generalized. In order to have a better insight on the effect of lipophilicity of the whole compound and not only the substituent (R) on antimycobacterial activity throughout the full series, Activity against *Mtb* H37Ra expressed as log (1/MIC) plotted against calculated lipophilicity log *P* (Figure 6). From the graph we find that the *in vitro* antimycobacterial activity against *Mtb* H37Ra was not directly correlated to calculated lipophilicity of compounds (log *P*). The most

active compounds were located both near the lower (JZ-AS8, JZ-AS9) and upper border of the log P interval. Similarly, inactive compounds were both of low (JZAS-1) and high lipophilicity (SB-1, JZAS-11).

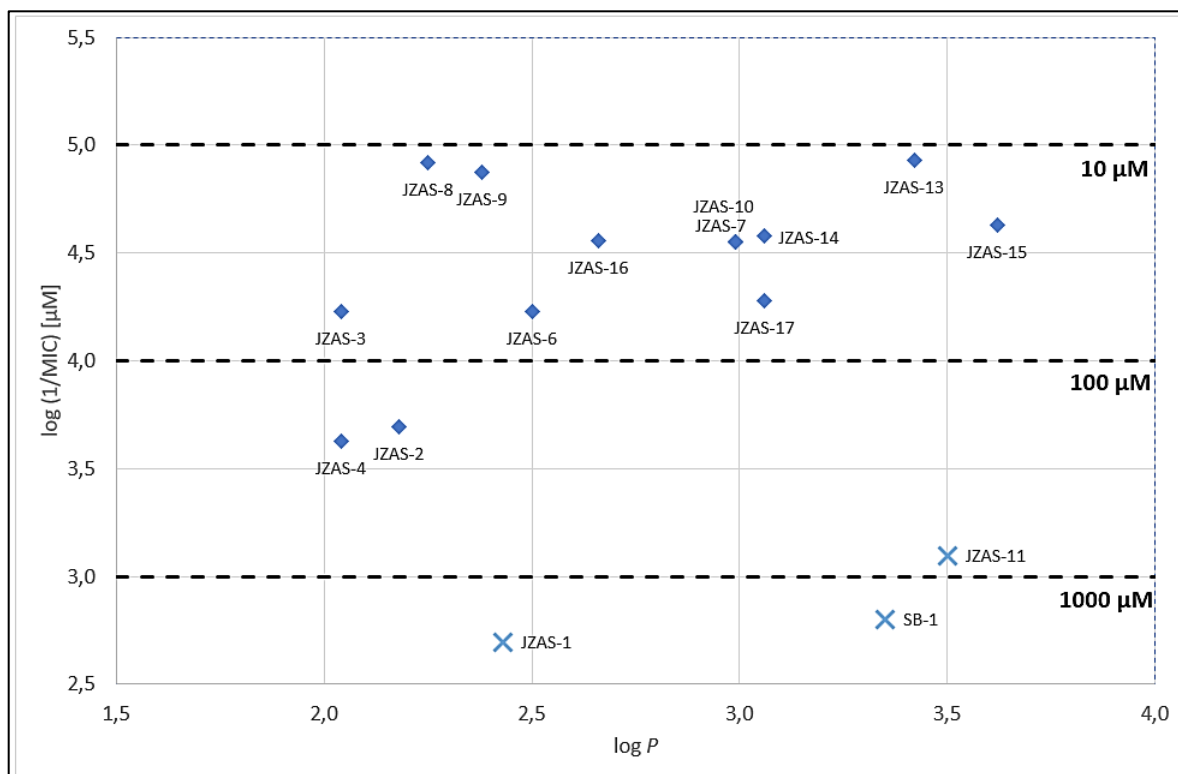


Figure 6: Activity against *Mtb* H37Ra expressed as log (1/MIC) plotted against calculated lipophilicity log P. ‘X’ mark denotes inactive compounds, whose actual activity is not known but lower then indicated by the mark.

According to the literature, antimycobacterial activity can be successfully *in vitro* evaluated against *Mtb* H37Rv or its avirulent strain *Mtb* H37Ra with comparable MIC values. However, in our case we found some discrepancies between the two values, for example the most active compound against *Mtb* H37Ra JZAS-13 was inactive against *Mtb* H37Rv, and therefore it would be necessary to retest all compounds.

On the other hand, no significant antimycobacterial activity was observed against *M. kansasii* and *M. avium*. Yet compounds JZAS-9 and JZAS-14 showed some activity against the two

latter strains, and beside their activity against *Mtb* H37Ra and *Mtb* H37Rv, they are considered to be the most promising compounds with broad spectrum of activity.

If we compare MIC values against *Mtb* H37Rv of some compounds from group B to structurally related compounds bearing the same substituent (R) previously prepared by our group, we find that those belonging to this series have inferior activity as shown in Figure 7.

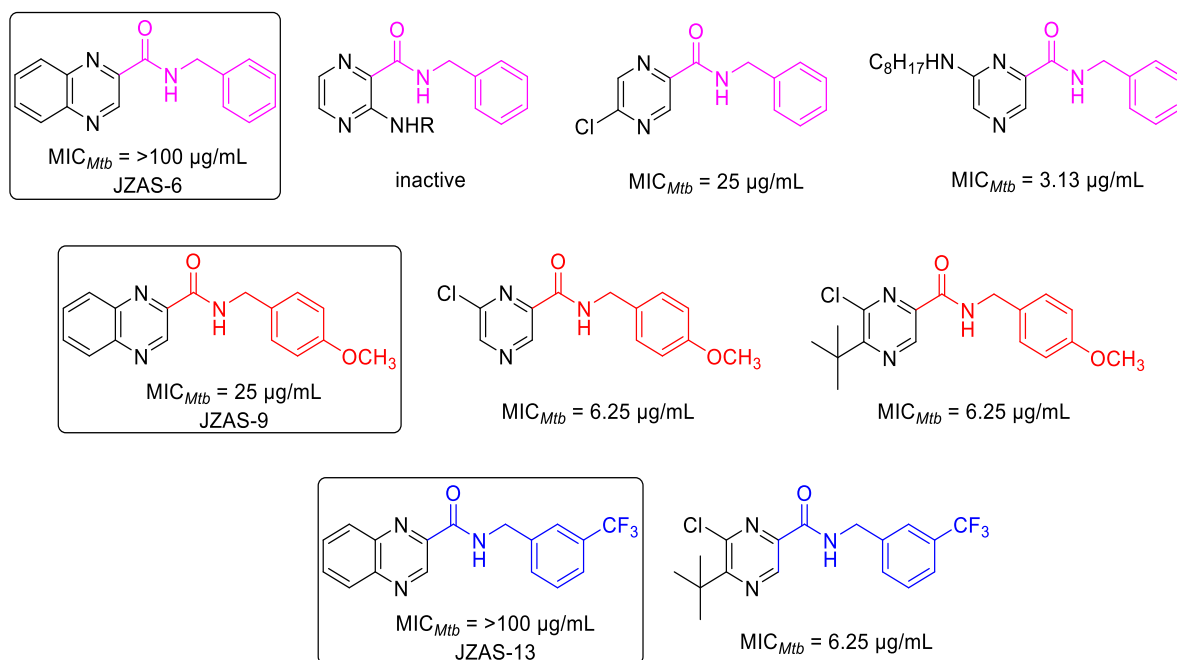


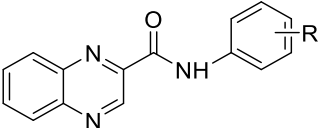
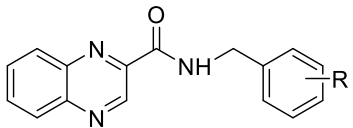
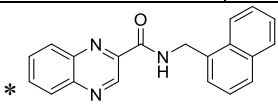
Figure 7: Comparison between antimycobacterial activity against *Mtb* H37Rv of selected compounds from group B (in rectangles) and their corresponding analogues previously prepared by our research group.

7.2. *In Vitro* Activity Evaluation Against *Mycobacterium smegmatis* and *Mycobacterium aurum*

All prepared compounds were tested against *M. smegmatis* and *M. aurum* as complementary screening. These two mycobacterial strains differ from the previous ones in being non-pathological and fast to grow.^[37] Despite these differences, drug susceptibility profile is somewhat common among all mycobacterial strains.^[38] Obtained biological evaluation results are summarized in Table 3. Again in agreement with antimycobacterial activity against the 4 previously mention strains in section 7.1., JZAS-14 was the most active against

M. smegmatis and *M. aurum* (MIC = 31.25 µg/mL), emphasising its broad spectrum of activity. JZAS-9 also exerted some activity against *M. aurum* (MIC = 125 µg/mL). JZAS-13 showed some activity against *M. smegmatis*. Beside these mentioned results, no significant antimycobacterial activity was detected.

Table 3: Prepared compounds with their antimycobacterial activity against *M. smegmatis* and *M. aurum* in µg/mL.

			
Cmpd.	R	<i>M. smegmatis</i>	<i>M. aurum</i>
JZAS-1	H	≥250	≥250
JZAS-3	3-OH	≥125	≥125
JZAS-4	4-OH	≥500	≥500
JZAS-2	3,5-diOCH ₃	≥125	≥125
SB-1	3-CF ₃	≥125	≥125
			
Cmpd.	R	<i>M. smegmatis</i>	<i>M. aurum</i>
JZAS-6	H	≥125	≥125
JZAS-7	2-CH ₃	≥125	≥125
JZAS-8	2,5-diOCH ₃	≥500	≥500
JZAS-9	4-OCH ₃	≥250	125
JZAS-13	3-CF ₃	250	≥500
JZAS-10	4-CH ₃	≥250	≥250
JZAS-11	*	≥125	≥125
JZAS-14	3-Cl	31.25	31.25
JZAS-16	3-F	≥500	≥500
JZAS-17	2-Cl	≥500	≥500
JZAS-15	3,4-diCl	≥500	≥500
			

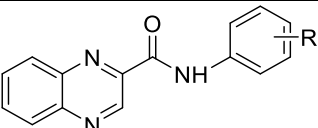
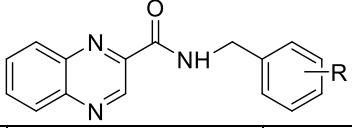
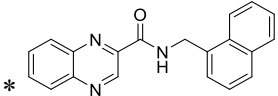
7.3. *In Vitro* Antibacterial Activity and Antifungal Evaluation

None of the prepared compounds exerted antibacterial or antifungal activity against tested strains (MIC >500 μ M).

7.4. *In Vitro* Cytotoxicity

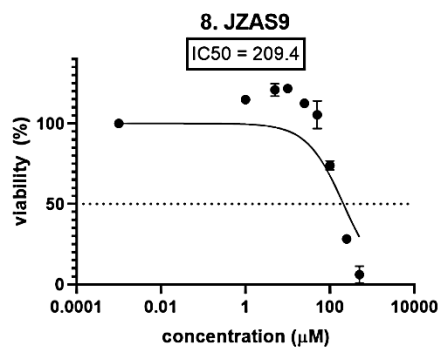
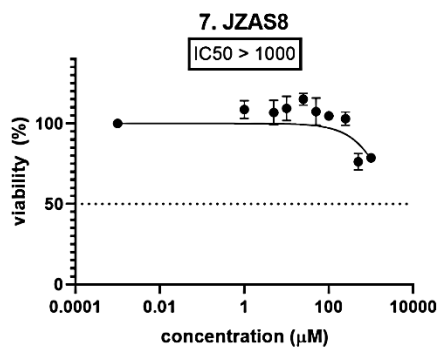
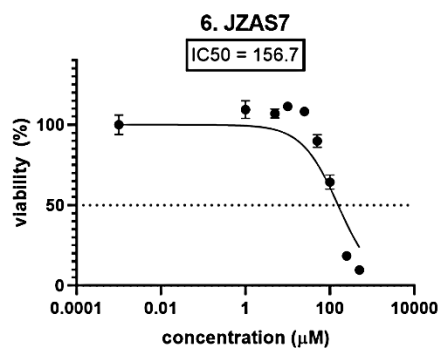
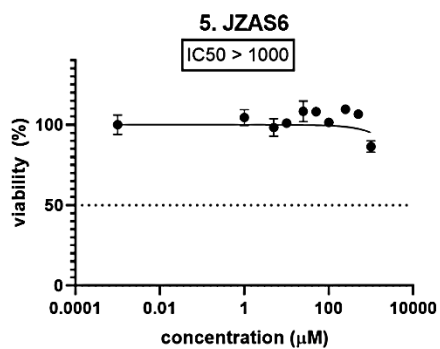
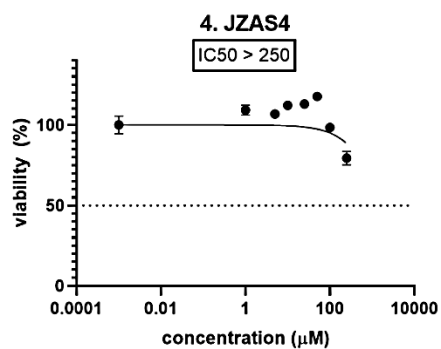
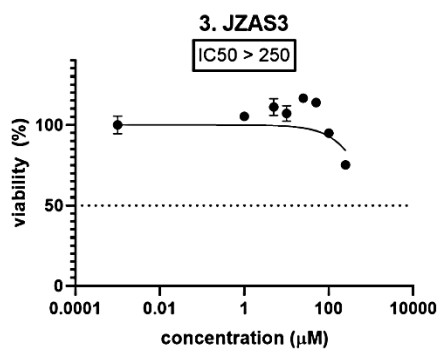
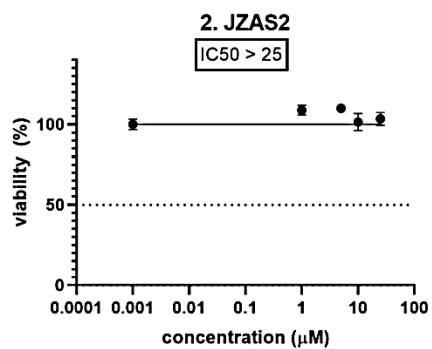
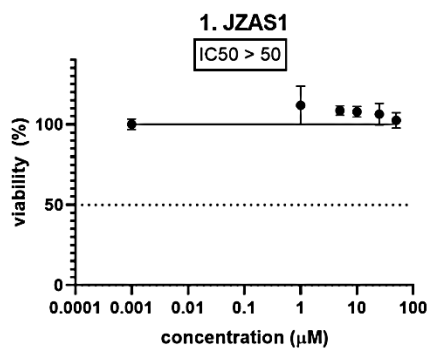
Cytotoxicity of the tested compounds was measured using the standard hepatic cell line HepG2. This particular cell line was chosen based on the fact that most available antituberculars exhibit a level of hepatotoxicity.^[39] The used CellTiter 96 assay is based on the reduction of tetrazolium of dye MTS in living cells to formazan, which is then determined colorimetrically. The reduction of the reagent is related to availability of NADH and NADPH. The decline in levels of these metabolically important compounds in the cell causes that the production of formazan is reduced. The parameter IC₅₀ was used as a measure of cytotoxicity, which allow the quantitative comparison of the toxicity among tested compounds. This parameter of toxicity was determined for the tested substances. All compounds belonging to the first structural group precipitated in the testing medium and therefore accurate measurement of IC₅₀ was not feasible. JZAS-6 and JZAS-8 are relatively nontoxic as IC₅₀ values are higher than the tested range of concentrations. Among the rest compounds, JZAS-10, JZAS-11, JZAS-13 and JZAS-15 exhibited relatively higher cytotoxicity than the other tested compounds. The major difference between the cytotoxicity of compounds JZAS-6 and JZAS-11 can be attributed to the presence of extra annulated benzene core (resulting in naphthyl substituent instead of phenyl). The effective cytotoxic concentrations of all tested compounds are listed in the Table 4. In order to evaluate the selectivity of prepared compounds SI was calculated as presented in Table 4 by dividing the IC₅₀ by the MIC in μ M against *Mtb* H37Ra. Selectivity index (SI) values above 10 indicates that the compound can be used at concentrations 10 times greater than its MIC without exerting toxicity.^[40] Based on obtained results of cytotoxicity and antimycobacterial activity discussed in sections 7.1. and 7.2., JZAS-9 is the most promising compound among all title compounds with broad spectrum of activity and SI value = 16.

Table 4: Cytotoxicity of the tested substances in HepG2 cells and their calculated selectivity index (SI) in μM against *Mtb* H37Ra.

			
Cmpd.	R	IC ₅₀ (μM)	SI
JZAS-1	H	>50**	0.02
JZAS-3	3-OH	>250**	4.20
JZAS-4	4-OH	>250**	1.01
JZAS-2	3,5-diOCH ₃	>25**	0.12
SB-1	3-CF ₃	>25**	0.02
			
Cmpd.	R	IC ₅₀ (μM)	SI
JZAS-6	H	>1000	17
JZAS-7	2-CH ₃	156.7	5.6
JZAS-8	2,5-diOCH ₃	>1000	82.6
JZAS-9	4-OCH ₃	209.4	16.1
JZAS-13	3-CF ₃	48.6	4.12
JZAS-10	4-CH ₃	87.7	3.11
JZAS-11	*	37.3	0.05
JZAS-14	3-Cl	140.1	5.34
JZAS-16	3-F	248	8.92
JZAS-17	2-Cl	527.5***	22.45
JZAS-15	3,4-diCl	n.a.	n.a.
			

** measurement at higher concentration was unable due to the precipitation of the tested compound in the cell culture medium

*** estimated value based on the curve



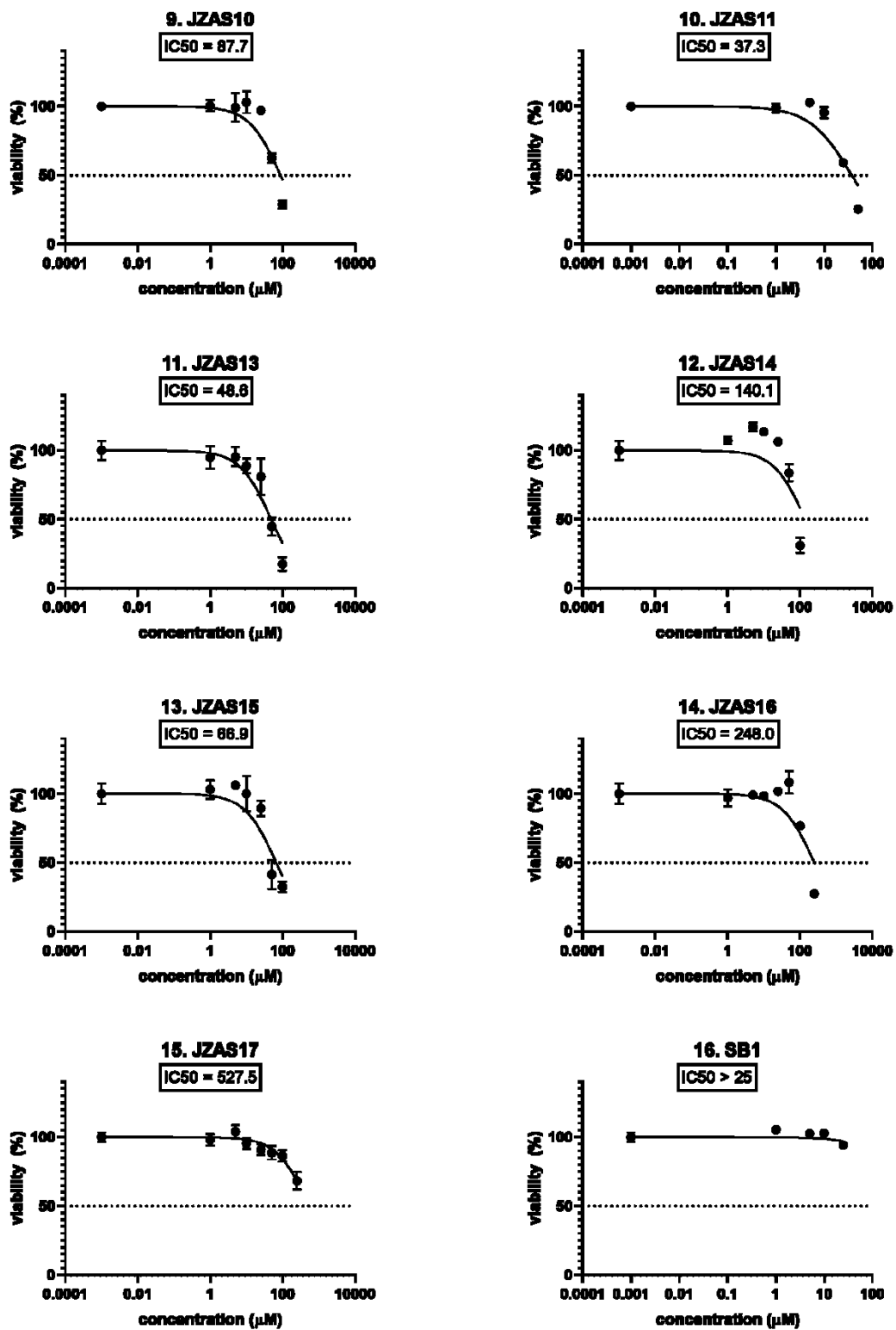


Figure 8: Cytotoxic effect of different incubation concentrations of the tested substances on HepG2 cells

8. CONCLUSION

To conclude, a total of 16 quinoxaline-2-carboxylic acid derivatives were synthesized and evaluated for their antimicrobial activity, out of which 14 compounds were new not previously described in literature (as of 20th of February, 2019). The 16 compounds were further subdivided into 5 *N*-phenylquinoxaline-2-carboxamides and 11 *N*-benzylquinoxaline-2-carboxamides. Compounds JZAS-1 and JZAS-6 were published as antidepressants but were not evaluated for antimicrobial activity. Most compounds showed moderate to strong antimycobacterial activity against *Mtb* H37Ra. The most active compounds with MIC = 3.91 µg/mL were JZAS-13 (11.8 µM), JZAS-8 (12.1 µM), and JZAS-9 (13 µM). *N*-Benzylquinoxaline-2-carboxamides had better activity than *N*-phenylquinoxaline-2-carboxamides. Compounds JZAS-9 and JZAS-14 showed broad spectrum of activity against mycobacterial strains, both fast and slow growing. Cytotoxicity evaluation against HepG2 liver cancer cell line favoured JZAS-9 as the most promising compound among all with selectivity index equal to 16. No antibacterial or antifungal activities were detected for any of tested compounds. In comparison to the antimycobacterial activity against *Mtb* H37Rv of previous pyrazine-2-carboxamides prepared by our group, quinoxaline core lowered the activity.

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