CORE
Metadata, citation and similar papers at core.ac.uk
Provided by IntechOpen

We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

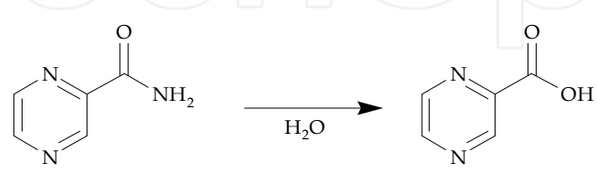
10

Pyrazinecarboxylic Acid Derivatives with Antimycobacterial Activity

Martin Doležal[1], Jan Zitko[1] and Josef Jampílek[2]
[1]*Faculty of Pharmacy in Hradec Králové, Charles University in Prague*
[2]*Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno*
Czech Republic

1. Introduction

Tuberculosis (TB) is still one of the major causes of bacterial infections and mortality in the world. In 1944, streptomycin was introduced as a first antibiotic remedy to cure TB effectively. After that, in the 1940s and 50s, with the introduction of pyrazinamide (PZA), rifampicin (RIF), isoniazid (INH), ethambutol and streptomycin as well as cocktail-drug treatment of tuberculosis, a cure for tuberculosis was considered reasonable. This period was a period of optimism, as it was believed that TB could be cured and eliminated. Hence, the United Nations targeted elimination of TB by 2025. These expectations were dashed as a worldwide pandemic of tuberculosis began in 1987 and the World Health Organization declared that tuberculosis posed a global emergency in 1993. The estimates of the global burden of the disease caused by *Mycobacterium tuberculosis* in 2009 are as follows: 9.4 million incident cases, 14 million prevalent cases, 1.3 million deaths among HIV-negative people and 0.38 million deaths among HIV-positive people. It is estimated that among TB patients notified in 2009, 250 000 had multidrug resistant TB (MDR-TB). The situation is worsening because of the increasing incidence of single-drug resistant, multidrug resistant and extensively drug-resistant (XDR-TB) TB (WHO report, 2010; The Stop TB Partnership, 2010; Velayati et al., 2009). This chapter deals with pyrazinecarboxamide (amide of pyrazinecarboxylic acid, PZA, see Fig. 1, structure **I**) and its derivatives. PZA was introduced to clinical practice in 1952, and it has been among the first-line antituberculosis (anti-TB) drugs (Kushner et al., 1952; Solotorovsky, 1952; Steele & Des Prez, 1988).



I **II**

Fig. 1. Hydrolysis of pyrazinamide (PZA, **I**) to pyrazinoic acid (POA, **II**).

PZA is an important component in the intensive phase of short-course treatment of TB owing to its sterilizing effect, ability to act in acidic environments and excellent synergy with rifampicin (Zhang & Mitchison, 2003). PZA exhibits stringent structure-activity

relationships (SAR) requiring the pyrazine ring and the carboxamide moiety (for more details see Section 2.3) for antimycobacterial activity. Pyrazine is a 6π-electron-deficient heteroaromatic compound, in which the inductive effects of the nitrogen atoms induce a partially positive charge on the carbon atoms. Pyrazine, its derivatives as well as other 1,4-diazines, *i.e.* compounds with partial pyrazine structure (e.g. quinoxaline, phenazine, pteridine, flavin and their derivatives), demonstrate unique physico-chemical properties that are caused by a low lying unoccupied π-molecular orbital and by the ability to act as a bridging ligand. Pyrazine and derivatives are known to provide a superexchange pathway for magnetic exchange (Awwadi et al., 2005). Due to these two properties 1,4-diazines, and especially their parent compound pyrazine, possess a characteristic reactivity. A fully comprehensive study of the pyrazines including reactivity and synthesis is beyond the scope of this work but can be found in the literature (Bird, 1992; Brown, 2002; Joule & Mills, 2010). Pyrazines are a class of compounds that occur almost ubiquitously in nature. Their effectiveness at very low concentrations and the ever increasing application of synthetic pyrazines in the flavour and fragrance industry and in the food and pharmaceutical industries are responsible for the high interest in these compounds (Doležal, 2006a). Tetramethylpyrazine (ligustrazine) was discovered as a natural product and is reported to scavenge the superoxide anion and decrease nitric oxide production in human polymorphonuclear leukocytes (Zhang, 2003). In plants or insects, pyrazines play the roles of attractants, pheromones and signal substances. Similar substances were found in food, and therefore their sensoric properties were investigated (Maga, 1992; Wagner et al., 1999). Mutagenic/carcinogenic pyrazines were found in processed foods, smoked-dried bonito meat, roasted coffee beans and charred eggs (Kikugawa, 2004). Synthetic pyrazines are used as "identical" additives in the food manufacturing and tobacco industries (Maga, 1992). Pyrazines are also synthesized by a number of fungi, such as the antibiotic aspergillic acid (White & Hill, 1943; Micetich & MacDonald, 1965) and the fungicidal pigment pulcherriminic acid (MacWilliam, 1959; MacDonald, 1965; Uffen Canole-Parola, 1972). The pyrazine derivative coelenterazine was found in many marine bioluminescent organisms (Thompson & Rees, 1994). Other marine pyrazine alkaloids botryllazines A and B isolated from the red ascidian *Botryllus leachi* possess interesting cytotoxic properties (Duran et al., 1999). The wide occurrence of pyrazines in nature has been already discussed (Woolfson & Rothschild, 1990; Müller & Rappert, 2010; Rajini et al., 2011). Pyrazines are also very important anthropogenic compounds, especially dihydropyrazines are essential for all forms of life due to their DNA strand-breakage activity and/or due to their influence on apoptosis (Yamaguchi, 2007). It is not surprising that many simple pyrazines have potent pharmaceutical activities. Examples are antiviral drugs flutimide (Singh & Tamassini, 2001), favipiravir (Furuta et al., 2009), the potential antineoplastic pyrazinediazohydroxide (Edelman et al., 1998) or acipimox, a niacin analogue used as a hypolipidemic agent (Ambrogi et al., 1980). The introduction of the pyrazine ring in bulky compounds brings specific chemical and physico-chemical properties for the whole molecule, like some weak basicity and aromatic character, examples are the antibacterial sulfonamide sulfadiazine (Roblin et al., 1940) or the second-generation sulfonylurea derivative with hypoglycaemic activity glipizide (Ambrogi, 1972). Such type of modification can be also found in the proteasome inhibitor for the treatment of multiple myeloma bortezomib (Adams, 1996), in the fentanyl derivative with selectivity for the μ opioid receptor mirfentanil (France et al.,

1991), in the drug for smoking cessation varenicline (Coe, 2005) or in the protease inhibitor for treatment of hepatitis C telaprevir (Furuta et al., 2009). Another examples are the diuretic amiloride and its derivative (Cragoe et al., 1964) newly also used for the treatment of cystic fibrosis, elpetrigine, a new chemical entity that combines two mechanisms known to work well in antiepileptic drugs (it is both a sodium channel and calcium channel blocker) (Foreman et al., 2008), and eszopiclone, a short acting nonbenzodiazepine sedative hypnotic (Cotrel et al., 1975). Diallylsulfide (a component of *Allium sativum*) has chemoprotective activity and pyrazine has binding affinity to cytochrome P-450 2E1 (inhibits CYP 2E1 activity), therefore, pyrazine was attached to the allylsulfide radical of diallylsulfide to form 2-allylsulfanylpyrazine to increase the binding affinity of diallylsulfide to CYP 2E1 (Kim et al., 1997). A similar pyrazine derivative, oltipraz, is an antischistosomal agent and a chemoprotective agent increasing the level of glutathione-*S*-transferases (Clapper, 1998). From this point of view the pyrazine ring can be an important part of peptide-mimicking molecules to modulate the interactions between proteins and a drug. The importance of the pyrazine (1,4-diazine) ring for biological activity can be evaluated primarily from the point of view of the molecular size/space and its physico-chemical properties. In relatively small compounds, the pyrazine ring is necessary for biological action due to its resemblance (bioisosterism) to the naturally occurring compounds (in human), for example, nicotinamide or anticancer and antiviral drugs containing the 1,4-diazine ring as position analogues of pyrimidine (1,3-diazine) nucleic bases (Doležal, 2006a).

Three main types of mycobacterial population are found in a patient with active TB, (*i*) actively growing bacilli, (*ii*) slowly growing bacilli inside macrophages, and (*iii*) the persisting semidormant bacilli localised extracellularly in solid caseous lesions. Since the pH is acidic inside the macrophages, PZA is the most active drug acting on this population followed by RIF, both drugs have crucial role in achieving sterilization (Mitchison, 1985; Zhang & Mitchison, 2003). While TB is curable, MDR-TB and XDR-TB may be fatal and the cure rates are frustratingly low. For this purpose, it is necessary to accelerate investigations to identify new types of anti-TB drugs acting on novel drug targets (Vinšová & Krátký, 2010).

2. Pyrazinamide

PZA (see Fig. 1, structure **I**) is indicated for the initial treatment of active tuberculosis in adults and children when combined with other anti-TB agents. PZA is an important sterilising tuberculosis drug that helps to shorten the duration of current chemotherapy regimens for tuberculosis. It is unique among antituberculosis drugs in having no genomic site of action and having greater bactericidal activity as bacillary metabolism slows down; it is remarkably effective in human disease. PZA is an important component in the intensive phase of short-course treatment of TB owing to its sterilising activity, ability to work in acidic environments (in macrophages), and excellent synergy with RIF (Mitchison, 1985; Zhang & Mitchison, 2003). Pyrazinamide appears to kill at least 95% of the semi-dormant bacterial population persisting in a low-pH environment since its activity is present only in the acidic environment found in active inflammation (Mitchison, 1985; Heifets et al., 1992). The development of a new drug with a similar mode of activity might be very fruitful, especially if there were no need for an acid environment (Aldrich et al., 2010). Objectives for

TB drug development are: (*i*) to shorten the total duration of effective treatment and/or significantly reduce the total number of doses needed to be taken under directly observed treatment, short-course (DOTS) supervision, (*ii*) to improve the treatment of MDR-TB, which cannot be treated with INH and RMP, and (*iii*) to provide a more effective treatment of latent TB infection (LTBI). Genomics, the systematic identification of all of the genes in a cell through deoxyribonucleic acid (DNA) sequencing and bioinformatic analysis, also offers great potential in terms of drug target discovery and development of new antibacterial agents, and the recently sequenced genome of *M. tuberculosis* should provide a number of new targets for novel drugs (Cole et al., 1998; Vinšová & Krátký, 2011).

2.1 Metabolism and toxicity of PZA

Pyrazine is a weak diacid base ($pK_1 = 0.57$; $pK_2 = -5.51$), weaker than pyridine (pyrazine $pK_a = 1.1$, pyridine $pK_a = 5.2$) due to the induction effect of the second nitrogen. The absorption of weak amine bases such as pyrazine derivatives is optimal at intestinal pH (5–7). In humans and laboratory rodents, orally administered substituted pyrazines are rapidly absorbed from the gastrointestinal tract and excreted. Methyl-substituted pyrazines are oxidized to yield the corresponding pyrazine-2-carboxylic acids. Pyrazine-2-carboxylic acid derivatives and 5-hydroxypyrazine-2-carboxylic acid derivatives are major urinary metabolites formed by side-chain oxidation and ring hydroxylation of alkyl-substituted pyrazine derivatives. PZA is hydrolyzed to pyrazine-2-carboxylic acid in humans and laboratory animals, while in other animals it can be metabolised also to 5-hydroxypyrazine-2-carboxylic acid (Sak-Bosnar & Kovar, 2005).

Early clinical studies described an unacceptable incidence of hepatotoxicity, but PZA exerts minimal toxicity when used for brief periods (preferably in six-month regimens) (Steele & Des Prez, 1988). The most frequent side effect is nongouty polyarthralgia (Zierski & Bek, 1980), and the phototoxicity of PZA has been described recently (Vargas et al., 2003).

2.2 Mechanism of action of PZA

The mode of action of PZA is unusual and has confused scientists for decades since the discovery of its antimycobacterial activity in 1952. The main reason is that PZA is completely different from common antibacterial chemotherapeutics that are primary active against growing bacteria, since PZA has no apparent activity against growing tubercle bacilli at neutral pH. PZA plays a unique role in shortening the therapy from 9–12 months to 6 months, because it kills a population of dormant/semi-dormant tubercle bacilli that are not killed by other antituberculous drugs (Mitchison, 1985). The basic knowledge about the mechanism of action of PZA was very poor for a long time. PZA as a prodrug form of pyrazinecarboxylic acid (pyrazinoic acid, POA, see Fig. 1, structure **II**) is devoid of significant antibacterial activity. PZA is activated in the mycobacterial cell by pyrazinamidase (PncA) and/or nicotinamidase (Konno et al., 1967), while its action is focused on depletion of membrane energy. Mycobacterial amidase, pyrazinamidase or nicotinamidase (nicotine deamidase, NAMase, EC 3.5.1.19), catalyzes the conversion of PZA to POA and nicotinamide to nicotinic acid (niacin) (Tarnok et al., 1979). POA is only active under acidic conditions (McDermott & Tompsett, 1954; Yamamoto et al., 1995). The cell envelope of *M. tuberculosis*, a Gram-positive bacterium with a G+ C-rich genome, contains

an additional layer beyond the peptidoglycan that is exceptionally rich in unusual free lipids, glycolipids and polysaccharides (Kolattukudy et al., 1997). The biosynthetic pathways generate cell-wall components such as mycolic acids, mycocerosic acid, phenolthiocerol, lipoarabinomannan and arabinogalactan, and several of these may contribute to mycobacterial longevity, trigger inflammatory host reactions and act in pathogenesis. The complete genome sequence of the strain *M. tuberculosis* H37Rv was determined (Cole et al., 1998). Although 40% of protein-coding genes were functionally annotated based on sequence similarity analysis and other analysis methods, 60% of them were reported as unknown functions (Manabe et al., 2000). This situation was improved in 2002 by Camus et al., who re-annotated *M. tuberculosis*'s genome (Camus, 2002). Mycolic acid is the major constituent of the unique mycobacterial cell wall, which comprises arabinogalactan-mycolate covalently linked with peptidoglycan and trehalose dimycolate and protects the tubercle bacillus from general antibiotics and from the host's immune system.

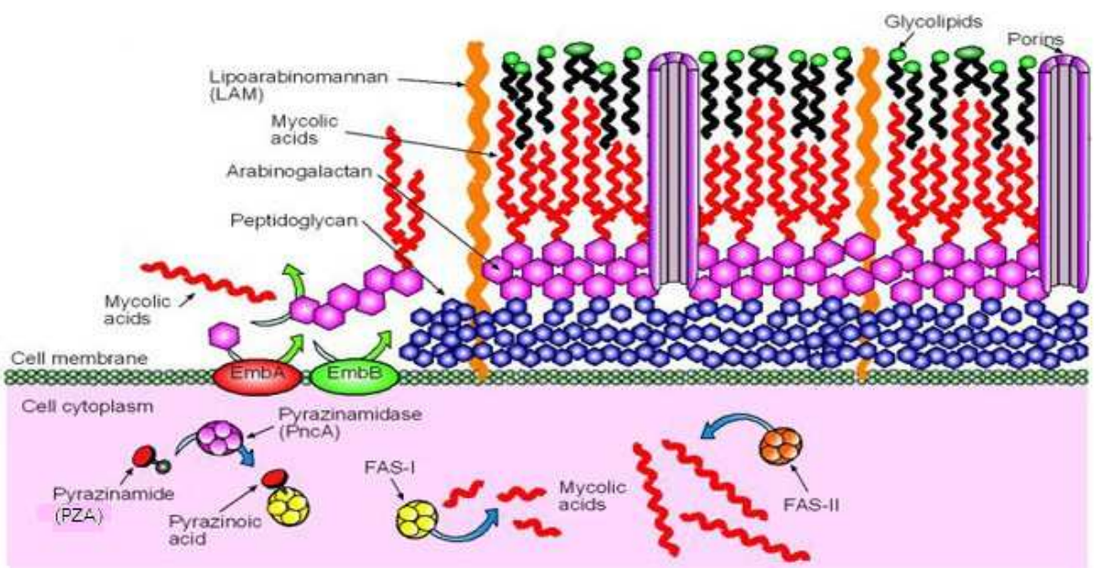


Fig. 2. Mode of action of PZA. The first-line antituberculosis drug PZA interferes with cell wall biosynthesis in *M. tuberculosis*. PZA is a prodrug and is converted to an active form (POA) by a nicotinamidase-peroxidase enzyme known as pyrazinamidase (PncA). POA inhibits the action of FAS I and FAS II. FAS I is involved in the synthesis of short-chain mycolic acids, and FAS II is involved in the synthesis of longer chains. Mycolic acids are essential structural components of the mycobacterial cell wall and are attached to the arabinogalactan layer. (Taken from Immunopeadia.org/First-Line Therapeutic Drugs in <http://www.immunopaedia.org.za/fileadmin/gallery/TB%20Drugs%20-%20Line/pyrazinamide.jpg> with the permission of the Research Administrator of the website.)

The synthesis of mycolic acids has been shown to be critical for the survival of *M. tuberculosis*; therefore, the mycolic acid pathway (MAP) has been of great interest as indicated by a large number of biochemical and genetic studies in the literature. The differences in the composition of free lipids at the cell envelope surface may result in strain-specific *M. tuberculosis-Homo sapiens* interplay modalities that contribute to determination of outcomes of the initial pathogen-host encounters as well as the infection

and disease course (Crick et al., 2008). Large fatty acids constitute a significant proportion of the mass of the cell wall core (see Fig. 2), forming a hydrophobic permeability barrier (Liu et al., 1996). The endogenous resistance of *M. tuberculosis* to many drugs (Brennan & Nikaido, 1995; Liu et al., 1999) is generally attributed to the presence of the permeability barrier generated by the mycolic acids. At least two discrete types of the enzyme system, fatty acid synthase I (FAS I, EC 2.3.1.85) and fatty acid synthetase II (FAS II), are involved in fatty acid biosynthesis in mycobacteria. The fatty acids are elongated by repeated cycles using these enzymes; generally the FAS I system is responsible for shorter chains (up to C_{16}–C_{26}) and the FAS II system, for longer chains found in meromycolates (to C_{56}) (Kolattukudy et al., 1997; Mdluli et al., 1998).

As PZA is a prodrug, hydrolytic activation by pyrazinamidase (PncA) that converts it to POA is required (Scorpio & Zhang, 1996). It was assumed that POA has both specific and nonspecific effects due to an intracellular accumulation of the liberated acid. This accumulation lowers the intracellular pH to a suboptimal level that is likely to inactivate a vital target enzyme (Zhang & Mitchison, 2003).

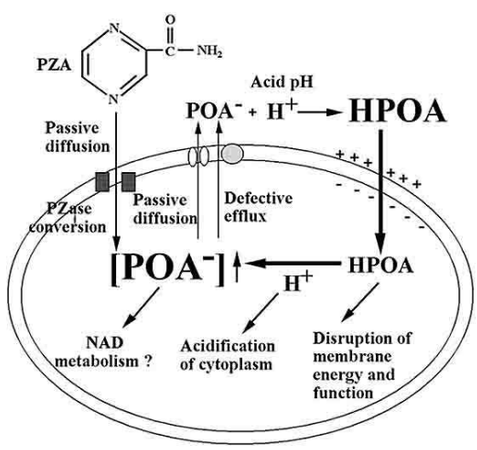


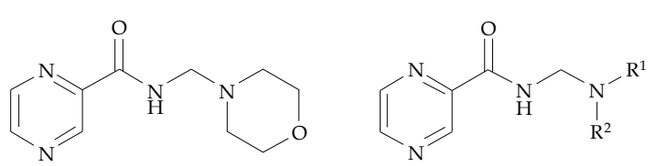
Fig. 3. Mode of action of PZA. PZA diffuses into *M. tuberculosis* in a passive transport, is converted into POA by PncA, and because of an inefficient efflux system, accumulates in huge amounts in the bacterial cytoplasm. (Ref. Wade & Zhang, 2004; reprinted with the editor's permission).

As pK_a of POA is 2.9, close to neutral intracellular pH, POA will be in the anion form and will be transported out of the cell through passive diffusion and deficient efflux to the cell surface (see Fig. 3). Under acidic pH, POA anion will form protonated or uncharged HPOA, which will diffuse back into the cell. The acid-facilitated POA influx is stronger than the weak POA efflux and this causes accumulation of POA in *M. tuberculosis* (Scorpio & Zhang, 1996). As HPOA enters the bacilli at acid pH, it brings in protons and over time could cause acidification of the cytoplasm and disruption of the proton motive force and depletion of energy. The disruption of proton motive force by POA then inhibits the membrane transport function. PZA acts as an ionophore, causing cellular acidification (Hurdle et al., 2011). Patient isolates that are resistant to PZA typically show mutation within the gene encoding PncA (Scorpio & Zhang, 1996). Some weak acids (benzoic, sorbic and propyl hydroxybenzoic acid), UV and various energy inhibitors were found to enhance the activity of PZA *in vitro* against *M. tuberculosis* (Wade & Zhang, 2006).

Raman et al. applied a systems-based approach, Flux Balance Analysis (FBA), to investigate the MAP of *M. tuberculosis* H37Rv for identification of antituberculosis drug targets in 2005 (Raman et al., 2005). The MAP model consists of four sub-pathways: (*i*) production of malonyl CoA, (*ii*) FAS I pathway, (*iii*) FAS II pathway and (*iv*) condensation of FAS I and FAS II products into methoxy- and keto-mycolic acids.

2.3 Modifications of PZA structure

The PZA and POA as potential analeptic drugs were synthesized in Germany (Dalmer & Eugen, 1936) and later in USA as intermediate compounds on the pathway of aminopyrazine synthesis (Hall & Spoerri, 1940), but the antimycobacterial activity of PZA was reported later, in 1952. The use of nicotinamide-related compounds for the therapy of tuberculosis followed the serendipitous observation that nicotinamide was effective for the treatment of murine tuberculosis (Chorine et al., 1945; McKenzie et al., 1949). The initial information that nicotinamide possessed modest antitubercular activity stimulated the evaluation of other analogues. This observation led to the subsequent discovery of not only PZA but also INH (Vinšová et al., 2008) and ethionamide. Of many nicotinamide analogues that were subsequently synthesized and evaluated for antituberculosis activity, only PZA was active *in vivo* (Deassau et al., 1952; Yeager et al., 1952; Malone et al., 1952; Solotorovsky et al., 1952). In the same year (Kushner et al., 1952) the synthesis of 49 PZA derivatives and 14 related compounds was reported, and their antimycobacterial activities in the *in vivo* murine system were briefly referred to, but no information on their *in vitro* antimycobacterial activities was provided. It was soon shown that an enzyme called nicotinamidase inside the mycobacterium hydrolyzed nicotinamide and pyrazinamide to the corresponding carboxylic acids, and these carboxylic acids were the actual active compounds (Konno et al., 1967). However, nicotinic and pyrazinoic acids did not demonstrate activity due to their poor penetration into mycobacterial cells. PZA is still unusual because of its narrow spectrum of activity. Several aminomethylene analogues/prodrugs of PZA were prepared and evaluated including morphazinamide (see Fig. 4, structure **III**), which is a morpholine derivative, Mannich base of PZA (Felder & Tiepolo, 1962); the lack of any demonstrable improvement over PZA *in vivo* led to its abandonment. Surprisingly, it was found that amides of 5-substituted pyrazinoic acid displayed good activity against *M. avium*, PZA-resistant *M. tuberculosis*, and phagocytized *M. tuberculosis*, and these compounds also showed activity over a broad range of pH values (Heifets et al., 1986). *M. avium* became a serious cause of disseminated infection among patients with AIDS. Novel aminomethylene analogues of PZA (see Fig. 4, general structure **IV**) were tested (Chung et al., 2008). The activity of *N*-(pyrrolidin-1-ylmethyl)pyrazine-2-carboxamide was higher than that of PZA when administered with rifalazil or RIF in a mouse model of infection. The activity of these analogues against PZA-resistant strains suggests that development of the second generation PZA analogues may be especially fruitful.



III **IV**

Fig. 4. Structures of aminomethylene analogues of PZA.

PZA exhibits remarkably stringent structure-activity relationships (SAR) demonstrating an absolute requirement for the pyrazine nucleus and the carboxamide moiety in position 2 for the activity. The modifications of the pyrazine ring with pyridazine and/or pyrimidine were not successful (Rodgers et al., 1952). It can be concluded that any isosteric replacement of carbon by nitrogen or shift of nitrogen to another position results in the loss of antimycobacterial activity. Also *ortho*-condensation of the aromatic/heteroaromatic ring with pyrazine nucleus did not lead to active compounds. Similarly, the substitution of the carboxamide group in thioamide, *N*-methyl, *N*-acetyl, hydrazide (analogy with INH), nitrile, tetrazole and free carboxylic acid provided compounds that were completely inactive *in vivo* (Kushner et al., 1952). One of the effective methods that can lead to new drug discovery is the bioisosteric replacement of a functional group. Numerous functional groups have been reported as bioisosteric replacements for the carboxylic acid functionality (Thornber, 1979). The prodrug approach in series of PZA derivatives is already very hopeful. Substituted pyrazinecarboxylic acid esters have been previously reported to have *in vitro* activity against *M. avium* and *M. kansasii* as well as *M. tuberculosis*. Modification of both the pyrazine nucleus and the ester functionality was successful in expanding the antimycobacterial activity associated with PZA to include *M. avium* and *M. kansasii*, organisms usually not susceptible to pyrazinamide. In an attempt to understand the relationship between the activities of the esters and the needed biostability, quantitative structure-activity relationships (QSAR) were found (Fernandes et al., 2010). While POA cannot pass through mycobacterial cell walls due to its low lipophilicity, the esterification of POA is a suitable approach to increase the likelihood of its penetration into the resistant mycobacteria (Cynamon et al., 1992; Cynamon et al., 1995). Thus series of POA esters were prepared and evaluated. PZA-resistant isolates became susceptible *in vitro* to pyrazinoic acid, and *n*-propyl pyrazinoate was the most promising candidate (see Fig. 5, structure **V**). Esters of POA appeared to circumvent the requirement for activation by mycobacterial amidase. The MICs of *n*-propyl pyrazinoate for *M. tuberculosis* isolates were lower than those of pyrazinoic acid. This may lead to a candidate compound with enhanced activity against both PZA-susceptible and PZA-resistant *M. tuberculosis* isolates suitable for clinical development (Cynamon et al., 1992; Speirs et al., 1995). However, efficacy studies in mice failed to show any antitubercular activity likely due to poor stability of the esters in plasma (Zhang & Mitchison, 2003). Another series of more lipophilic ester prodrugs (*i.e.* tetradecyl ester) were found to be active in concentrations 10-fold lower than those needed for PZA to kill sensitive *M. tuberculosis* and also have suitable stability in the presence of plasma (Simoes et al., 2009). These relationships are consistent with the observation that *tert*-butyl 5-chloropyrazine-2-carboxylate and 2-methyldecyl 5-chloropyrazine-2-carboxylate are 100-fold more active than PZA against *M. tuberculosis* and exhibit serum stability 900-1000 times greater than the lead compounds in the series. Some 5-hydroxypyrazine-2-carboxylic acid derivatives (see Fig. 5, general structure **VI**) are up to 1000-fold more active against *M. tuberculosis* and other *Mycobacterium* strains than existing antituberculous agents (Bergmann et al., 1996); synthesis of compounds **VI** is important, because it is a building block for the synthesis of new antituberculous agents. 5-Hydroxypyrazine-2-carboxylic acid can be produced microbiologically by whole-cell biotransformation of 2-cyanopyrazine (Wieser et al., 1997). Various substituted

analogues of PZA with bioisosteric replacements of the carboxylic acid functionality were prepared. Thus 5-aroylpyrazine-2-carboxylic acid (see Fig. 5, general structure **VII**, Doležal et al., 2003) and arylsulfanylpyrazinecarboxylic acid (see Fig. 5, general structure **VIII**, Jampílek et al., 2007) derivatives were investigated as potential antitubercular agents.

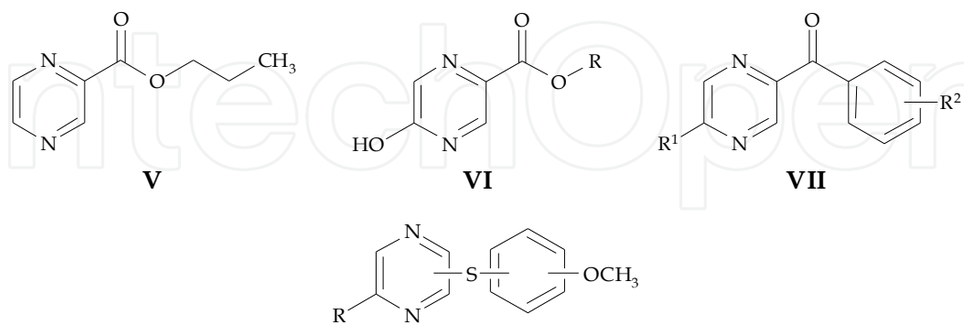


Fig. 5. Structures of pyrazine-2-carboxylic acid derivatives.

In order to find more active PZA derivatives, various PZA analogues were synthesized and assayed against *M. tuberculosis* (Yamamoto et al, 1995). In these experiments, four compounds showed high levels of antimycobacterial activities, not only bacteriostatic but also bactericidal, against *M. tuberculosis* as well as *M. avium* complex (MAC). These compounds, namely, pyrazinoic acid pivaloyloxymethyl ester, pyrazinoic acid *n*-octyl ester, pyrazinethiocarboxamide and *N*-hydroxymethylpyrazinethiocarboxamide, may warrant further examinations. 5-Chloropyrazine-2-carboxamide (see Fig. 6, structure **IX**) showed excellent *in vitro* activity against PZA-resistant strains of *M. tuberculosis* (Cynamon et al., 1998). Therefore FAS I and/or FAS II were proposed as a target of this compound, *i.e.* this compound possesses a different mechanism of action (Boshoff et al., 2002). Due to this fact 3-chloropyrazine-2,5-dicarbonitrile (see Fig. 6, structure **X**, Palek et al., 2008) and 6-chloro-5-cyanopyrazine-2-carboxamide (see Fig. 6, structure **XI**, Dlabal et al., 1990; Zitko et al., 2011) and their derivatives were synthesized, and their noteworthy antimycobacterial activities were reported recently.

3. Anti-*Mycobacterium tuberculosis* bioassays

Several *in vitro* bioassays have been developed to evaluate antitubercular activity of chemical compounds. In most of these methods, *Mycobacterium* is cultured in various types of broth- and agar-based media. But, the main problems are long growth time (several weeks) and its pathogenicity, hence containment facilities are required. The common conditions for the anti-TB susceptibility evaluation are influenced by acid-base properties of medium (McDermott & Tompsett, 1954). For nearly two decades the radiometric BACTEC 460TB System provided the most rapid method for antimicrobial susceptibility testing (Siddiqi, 1992).

The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) was established in 1994 by the National Institute of Allergy and Infectious Diseases (a part of the National Institute of Health of the US Government) in order to encourage the discovery of

new antitubercular drugs. The program managed by Southern Research Institute (Birmingham, Alabama, USA) assisted commercial, academic and government laboratories worldwide in identifying new chemical classes of compounds for evaluation using *in vitro* and *in vivo* models. These screening activities have resulted in several promising agents that have reached advanced stages of testing. TAACF was providing a great service to mankind by way of screening thousands of compounds synthesized in research laboratories across the globe for activity against TB and related diseases. The TAACF program has ended in March 2010 (TAACF, 2010). This *in vitro* screening had several levels.

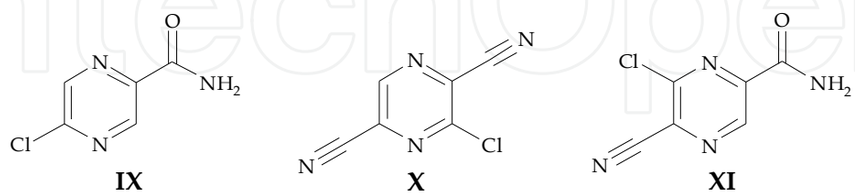


Fig. 6. Structures of chloropyrazine derivatives.

3.1 *In vitro* bioassays (% of inhibition)

The primary screening was conducted at 6.25 μg/mL (or molar equivalent of the highest molecular-weight compound in a series of congeners) against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) (Collins & Franzblau, 1997). Compounds **1–91** and **104–115** were tested in the BACTEC 460-radiometric system. In general, compounds effecting >90% inhibition in the primary screening (MIC <6.25 μg/mL) were further evaluated. The results are presented in Tables 1, 2 and 4.

3.2 Primary screening (dose response): Determination of 90% inhibitory concentration (IC_{90} values)

The initial screening was conducted against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) (Collins & Franzblau, 1997). Compounds **92-103** were tested in ten 2-fold dilutions; typically from 100 μg/mL to 0.19 μg/mL. The IC_{90} is defined as the concentration effecting a reduction in fluorescence of 90% relative to controls. This value is determined from the dose-response curve using a curve-fitting programme. Any IC_{90} value of ≤10 μg/mL is considered "active" for antitubercular activity. For the results see Table 3.

3.3 Secondary screening: Determination of mammalian cell cytotoxicity (CC_{50})

The VERO cell cytotoxicity assay was done in parallel with the TB Dose Response assay. After 72 hours exposure, viability was assessed using Promega's Cell Titer Glo Luminescent Cell Viability Assay, a homogeneous method of determining the number of viable cells in culture based on quantification of the ATP present. Cytotoxicity was determined from the dose-response curve as the CC_{50} using a curve fitting program. Ultimately, the CC_{50} was divided by the IC_{90} to calculate an SI (Selectivity Index) value. SI values of ≥10 were considered for further testing (TAACF, 2010). The results are presented in Table 2.

4. Physico-chemical properties of prepared compounds

It is generally admitted in drug research that the passage of molecules across cellular barriers increases with lipophilicity and that the most lipophilic compounds have the highest intestinal absorption. Most of the drugs behave in solution as weak acids, weak bases or sometimes as both weak acids and weak bases.

4.1 Capacity factor *k* determination (log *k* and distributive parameters π)

A Waters Alliance 2695 XE HPLC separation module and a Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, USA) were used. A Symmetry® C18 5 µm, 4.6×250 mm (Waters Corp., Milford, MA, USA) chromatographic column was used. The HPLC separation process was monitored by Millennium32® Chromatography Manager Software, Waters 2004 (Waters Corp., Milford, MA, USA). A mixture of MeOH p.a. (70.0%) and H_2O-HPLC – Mili-Q Grade (30.0%) was used as a mobile phase. The total flow of the column was 1.0 mL/min, injection volume 30 µL, column temperature 30 °C and sample temperature 10 °C. The detection wavelength of 223 nm was chosen. The KI methanolic solution was used for the dead time (t_D) determination. Retention times (t_R) were measured in minutes, capacity factors (*k*) were calculated. The calculated log *k* values of all compounds are shown in Tables 1, 3 and 4.

The distributive parameters π characterizing lipophilicity of individual moieties substituted on the skeleton were calculated as the differences of determined capacity factor logarithms of individual unsubstituted and substituted compounds. The calculated π values of all substituents in the amide part of the molecule are shown in Tables 1, 3 and 4.

4.2 Physico-chemical properties calculations (log *P*, log *S*, pK_a)

For calculation of the basic parameters the ACD/Labs software was used. The values of hydro/lipophilic properties expressed as log *P* were calculated by ACD/LogP DB; solubility data expressed as log *S* were calculated by ACD/LogS DB (aqueous log *S* at pH 7.4), and acid-base characteristics expressed as dissociation constants pK_a were calculated by ACD/pK_a DB. Results are shown in Tables 1, 3 and 4.

5. Antimycobacterial evaluation of substituted pyrazinecarboxamides

The amide function considered in this chapter is based on the bivalent moiety -CONH-, which can form centrosymmetric dimer pairs: (*i*) with another molecule of carboxamide (see Fig. 7, a) or (*ii*) with the carboxamide group of some peptide (see Fig. 7, b). Various compounds possessing -CONH- moiety as a bridging ligand between the heteroaromatic or basic part and the aromatic part of the molecule were found to inhibit the *M. tuberculosis*. The presented study deals with the synthesis of the series of amides prepared *via* anilinolysis of substituted pyrazinecarboxylic acid chlorides with alkylated, hydroxylated and/or halogenated anilines (see Section 5.1) or with substituted aminothiazoles (see Section 5.2). The aim of this work is to find the structure-activity relationships in the mentioned series, *i.e.* to continue studying the substituent variability influence on the biological activity, and to determine the importance of increased hydrophobic properties for biological evaluation of newly prepared substituted pyrazinecarboxamides.

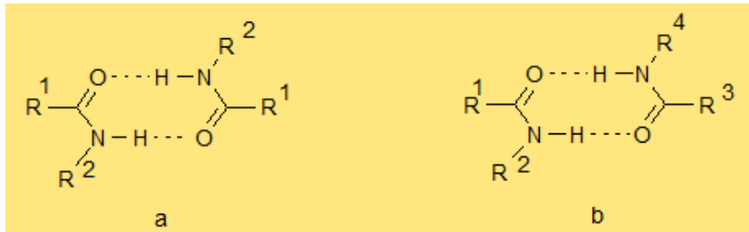


Fig. 7. Possible formation of centrosymmetric dimer pairs of carboxamide group.

5.1 Substituted *N*-phenylpyrazine-2-carboxamides

An effective method that can lead to design of new drugs with higher or modified biological activity is analogy application. Pyrazine skeleton can be substituted by aliphatic, cyclic or hetero/aromatic substituents. Also hydrogens of amidic nitrogen can be substituted. It is important to note that an amidic bond (-CONH-) can mimic a peptide bond. This moiety can form hydrogen bonds to active sites of various enzymes, and therefore only one amide hydrogen should be substituted to maintain this bonding capability (namely by an aromatic (phenyl) or heteroaromatic ring in this series). This approach (radical analogy) provides multiple opportunities for further modifications; therefore a series of 115 carboxamides derived from pyrazine-2-carboxylic acid and various ring-substituted anilines and/or 2-aminothiazoles was designed and prepared. These compounds were primarily designed as potential antimycobacterial compounds, but they also showed noteworthy antifungal activity. The assumption concerning binding to different enzymes through the amide moiety was confirmed, as the mentioned compounds considerably inhibited electron transport in photosystem PS 2 during photosynthesis in spinach chloroplasts, so they can be potentially used as herbicides (Doležal & Králová, 2011).

The final compounds **1-103** were prepared by the anilinolysis of substituted pyrazinoylchlorides (Doležal et al., 1999, 2000, 2002, 2006b, 2007, 2008a, 2008b, 2009, 2010; their synthesis and structure are presented in Fig. 8 and in Tables 1 and 3). Their chemical structure, electronic parameters expressed as Hammett's σ parameter (Norrington et al., 1975), hydrophobic parameters (experimentally determined log *k*, experimentally determined distributive parameters π_{Ph} for individual aniline substituents, log *P* calculated by ACD/LogP DB), solubility (log *S* calculated by ACD/LogS DB), dissociation constants (pK_a calculated by ACD/pK_a DB), antituberculotic inhibiting activity and structure-activity relationships (SAR) were studied.

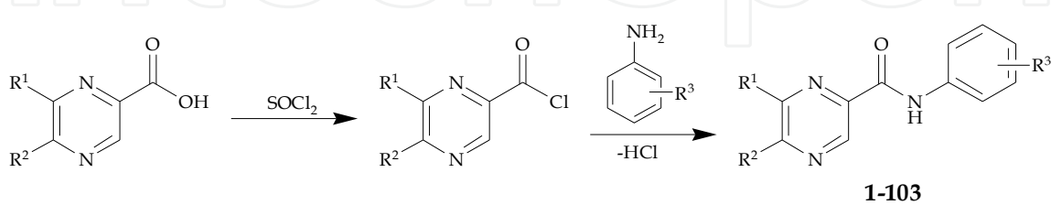


Fig. 8. Synthesis and structure of substituted *N*-phenylpyrazine-2-carboxamides **1-103**.

The following was synthesized in preference: (*i*) compounds with lipophilic and/or electron-withdrawing substituents on the benzene moiety (R^3), (*ii*) compounds with hydrophilic and/or electron-donating groups on the benzene part of the molecule (R^3), and

finally (*iii*) compounds with a lipophilic alkyl chain (R^2), *i.e. tert*-butyl (-$C(CH_3)_3$) and/or halogen (chlorine) substitution (R^1) on the pyrazine nucleus. The data are presented in Tables 1 and 3. The substituents on the phenyl ring were selected according to the principles set by Topliss (Topliss & Costello, 1972).

No.	R^1	R^2	R^3	Inhib. [%]	σ_{Ph}	π_{Ph}	log *k*	log *P*	log *S*	pK_a	Ref.
PZA	**see Fig. 1, structure I**			**	–	–	–	**-0.71±0.28**	**-0.76**	**13.91±0.50 0.87±0.10**	**
1	H	H	3-CF_3	**99**	0.43	0.19	0.5585	3.05±0.54	-3.23	11.36±0.70 0.14±0.10 -4.48±0.10	b
2	Cl	$(CH_3)_3C$	4-OH	**97**	-0.37	-0.21	0.9236	3.15±0.95	-3.72	11.34±0.30 9.50±0.70 1.30±0.50 -4.79±0.10	f
3	Cl	$(CH_3)_3C$	2-OH	**96**	-0.09	-0.08	1.1148	4.04±0.69	-4.04	11.46±0.35 8.52±0.35 1.57±0.50 -4.88±0.10	f
4	Cl	$(CH_3)_3C$	3-CF_3	**95**	0.43	0.19	1.5271	5.03±0.68	-5.10	9.44±0.70 -0.25±0.10 -4.87±0.10	b
5	Cl	$(CH_3)_3C$	3-F	**88**	0.34	0.03	1.2974	3.20±0.71	-4.35	9.20±0.70 -0.30±0.10 -4.92±0.10	d
6	H	H	4-CH_3	**86**	-0.17	0.07	0.4595	2.70±0.50	-2.68	11.91±0.70 0.80±0.50 -4.27±0.10	b
7	Cl	H	3-CF_3	**77**	0.43	0.19	0.8362	3.44±0.67	-3.88	9.37±0.70 -0.84±0.50	b
8	Cl	$(CH_3)_3C$	3-OH	**75**	0.12	-0.19	0.9473	3.24±0.65	-3.76	10.54±0.10 9.09±0.10 -0.13±0.10 -4.67±0.10	f
9	Cl	H	4-$CH(CH_3)_2$	**73**	-0.15	0.37	0.9920	3.64±0.64	-3.90	9.88±0.70 0.61±0.50	d
10	Cl	$(CH_3)_3C$	4-OH-3,5-Br	**72**	0.41	0.50	1.8895	5.25±1.03	-5.34	8.84±0.70 6.56±0.36 -0.38±0.50	a
11	Cl	$(CH_3)_3C$	3,5-CF_3	**72**	0.86	0.51	1.8982	5.92±0.74	-5.95	8.90±0.70 -0.40±0.10	e
12	Cl	H	4-CH_3	**71**	-0.17	0.07	0.7627	3.10±0.64	-3.34	9.92±0.70 0.64±0.50	b
13	H	$(CH_3)_3C$	3-F	**71**	0.34	0.03	0.9588	2.91±0.59	-3.75	9.87±0.70 1.07±0.10 -3.55±0.10	d
14	H	$(CH_3)_3C$	4-$CH(CH_3)_2$	**71**	-0.15	0.37	1.3281	4.94±0.51	-4.51	10.62±0.70 1.31±0.10 -3.29±0.10	d

No.	R^1	R^2	R^3	Inhib. [%]	σ_{Ph}	π_{Ph}	log *k*	log *P*	log *S*	pK_a	Ref.
15	Cl	H	4-OH-3,5-Br	**69**	0.41	0.50	1.1350	3.66±1.02	5.34	8.76±0.70 6.55±0.36 -0.39±0.50	a
16	Cl	H	4-Cl	**65**	0.23	0.16	0.8185	2.23±0.73	-3.37	9.04±0.70 -0.53±0.50	g
17	Cl	H	3,4-Cl	**61**	0.60	0.38	0.9950	3.60±0.81	-4.38	8.37±0.70 -1.33±0.50	a
18	H	$(CH_3)_3C$	4-CH_3	**61**	-0.17	0.07	1.0222	4.40±0.52	-3.98	10.66±0.70 1.32±0.10 -3.29±0.10	b
19	H	$(CH_3)_3C$	4-OH-3,5-Br	**54**	0.41	0.50	1.4758	4.96±0.95	-4.74	9.50±0.70 6.64±0.36 0.88±0.10 -3.75±0.10	a
20	H	$(CH_3)_3C$	3-OCH_3	**53**	0.12	-0.02	0.9146	3.71±0.52	-3.71	10.19±0.70 1.28±0.10 -3.34±0.10	a
21	H	$(CH_3)_3C$	4-$COCH_3$	**53**	0.81	-0.06	0.8573	3.34±0.53	-3.92	10.12±0.70 0.98±0.10 -3.45±0.10	h
22	H	$(CH_3)_3C$	4-OH	**50**	-0.37	-0.21	0.6779	2.86±0.87	-3.12	11.39±0.30 9.64±0.26 1.40±0.10 -3.42±0.10	f
23	Cl	H	4-F	44	0.06	0.05	0.7284	2.14±0.69	-3.29	8.89±0.70 0.19±0.50	b
24	H	$(CH_3)_3C$	3-OH	44	0.12	-0.19	0.7130	2.95±0.51	-3.15	10.79±0.70 9.16±0.10 1.24±0.10 -3.30±0.10	f
25	H	$(CH_3)_3C$	2,4-F	44	0.53	0.14	1.0992	2.99±0.68	-4.24	8.55±0.70 0.90±0.10 -3.73±0.10	d
26	H	$(CH_3)_3C$	3-Br	39	0.39	0.21	1.1516	4.45±0.57	-4.28	9.98±0.70 1.19±0.10 -3.43±0.10	e
27	H	H	2-CF_3	31	–	0.30	0.6511	2.60±0.54	-3.07	11.14±0.70 0.04±0.10 -4.59±0.10	b
28	Cl	$(CH_3)_3C$	2-OH-5-Br	30	0.30	0.17	1.5181	5.27±0.74	-4.92	10.80±0.48 7.86±0.48 0.73±0.50 -4.94±0.10	a
29	Cl	H	2-Br	28	0.71	0.40	1.0014	2.67±0.69	-3.47	8.98±0.70 -0.83±0.50	a
30	Cl	H	3-F	28	0.34	0.03	0.7135	1.61±0.70	-3.1	9.13±0.70 -0.65±0.50	d
31	Cl	H	3-CH_3	28	-0.07	0.07	0.7557	3.13±0.64	-3.35	9.92±0.70 0.24±0.50	e

No.	R^1	R^2	R^3	Inhib. [%]	σ_{Ph}	π_{Ph}	log *k*	log *P*	log *S*	pK_a	Ref.
32	H	$(CH_3)_3C$	2-OH	28	-0.09	-0.08	0.8350	3.76±0.57	-3.44	11.51±0.35 8.67±0.35 1.63±0.50 -3.51±0.10	f
33	Cl	H	2-CF_3	26	–	0.30	0.9102	3.00±0.67	-3.72	9.15±0.70 -1.33±0.10	b
34	Cl	$(CH_3)_3C$	2-Br	25	0.71	0.40	1.7673	4.26±0.70	-4.71	9.06±0.70 -0.39±0.10	a
35	H	$(CH_3)_3C$	4-F	25	0.06	0.05	0.9948	3.44±0.57	-3.94	9.63±0.70 1.20±0.10 -3.42±0.10	b
36	Cl	$(CH_3)_3C$	3-CH_3	24	-0.07	0.07	1.3288	4.71±0.65	-4.59	9.99±0.70 0.25±0.50 -4.57±0.10	e
37	Cl	$(CH_3)_3C$	4-Cl	24	0.23	0.16	1.5015	3.82±0.78	-4.62	9.11±0.70 -0.26±0.10 -4.88±0.10	g
38	H	$(CH_3)_3C$	3-CF_3	23	0.43	0.19	1.1338	4.74±0.55	-4.50	10.11±0.70 1.12±0.10 -3.50±0.10	b
39	Cl	$(CH_3)_3C$	3-OCH_3	23	0.12	-0.02	1.2148	4.00±0.65	-4.31	9.53±0.70 -0.09±0.10 -4.70±0.10	a
40	H	H	2-OH-5-Cl	22	0.28	0.12	0.4541	3.71±0.75	-3.23	11.45±0.70 8.12±0.48 0.93±0.50 -4.67±0.10	f
41	H	$(CH_3)_3C$	2-Br	22	0.71	0.40	1.4057	3.97±0.57	-4.11	9.72±0.70 0.98±0.10 -3.64±0.10	a
42	Cl	$(CH_3)_3C$	4-$COCH_3$	22	0.81	-0.06	1.1676	3.63±0.66	-4.52	9.46±0.70 -0.39±0.10 -4.82±0.10	h
43	Cl	$(CH_3)_3C$	2,4-F	21	0.53	0.14	1.4793	3.28±0.78	-4.84	7.88±0.70 -0.05±0.50	d
44	Cl	$(CH_3)_3C$	3-Br	20	0.39	0.21	1.5502	4.73±0.70	-4.88	9.32±0.70 -0.18±0.10 -4.80±0.10	e
45	Cl	H	3-Br	19	0.39	0.21	0.8452	3.15±0.69	-3.64	9.24±0.70 -0.75±0.50	e
46	H	$(CH_3)_3C$	2-OCH_3	18	0.00	0.04	0.9928	4.17±0.56	-3.87	10.02±0.70 1.28±0.10 -3.34±0.10	e
47	Cl	$(CH_3)_3C$	2-OH-5-Cl	17	0.28	0.12	1.4529	5.69±0.87	-5.14	10.93±0.48 7.99±0.48 0.78±0.50	f
48	Cl	H	2-Cl-5-OH	15	0.79	0.11	0.7801	1.78±0.69	-3.06	10.09±0.18 8.60±0.70 -1.09±0.50	b

No.	R^1	R^2	R^3	Inhib. [%]	σ_{Ph}	π_{Ph}	log *k*	log *P*	log *S*	pK_a	Ref.
49	H	$(CH_3)_3C$	3,4-Cl	15	0.60	0.38	1.3395	4.90±0.78	-5.01	9.11±0.70 0.83±0.10 -3.80±0.10	a
50	Cl	$(CH_3)_3C$	4-F	15	0.06	0.05	1.3238	3.72±0.70	-4.54	8.97±0.70 0.19±0.50 -4.79±0.10	b
51	H	H	3-Cl	14	0.37	0.14	0.4914	3.24±0.65	-3.22	11.24±0.70 0.09±0.10 -4.53±0.10	g
52	Cl	H	3-Cl	14	0.37	0.14	0.7864	3.63±0.77	-3.88	9.24±0.70 -0.71±0.50	g
53	Cl	H	4-OH	14	-0.37	-0.21	0.5587	1.56±0.95	-2.46	11.33±0.30 9.43±0.70 1.30±0.50	f
54	Cl	H	3-OH	13	0.12	-0.19	0.5758	1.65±0.64	-2.49	10.54±0.10 9.08±0.10 -0.17±050	f
55	Cl	$(CH_3)_3C$	2,6-CH_3	13	0.20	0.20	1.5286	4.23±0.66	-4.56	10.00±0.70 1.45±0.50 -4.74±0.10	e
56	Cl	H	3,5-CF_3	12	0.86	0.51	1.1384	4.33±0.71	-4.77	8.82±0.70 -1.39±0.10	e
57	Cl	H	4-$COCH_3$	11	0.81	-0.06	0.6548	2.04±0.65	-3.29	9.38±0.70 -1.37±0.10	h
58	Cl	H	3,5-OCH_3	11	0.24	0.08	0.7701	2.70±0.66	-3.32	8.99±0.70 -0.43±0.50	a
59	H	$(CH_3)_3C$	3-CH_3	11	-0.07	0.07	1.0191	4.43±0.52	-3.99	10.65±0.70 1.41±0.10 -3.20±0.10	e
60	H	$(CH_3)_3C$	3,5-CF_3	10	0.86	0.51	1.4861	5.63±0.62	-5.35	9.56±0.70 0.96±0.10 -3.66±0.10	e
61	H	H	3,4-Cl	8	0.60	0.38	0.7162	3.20±0.78	-3.72	10.36±0.70 -0.15±0.10 -4.78±0.10	g
62	H	$(CH_3)_3C$	2-CF_3	7	–	0.30	1.2522	4.30±0.55	-4.34	9.89±0.70 1.02±0.10 -3.61±0.10	b
63	H	$(CH_3)_3C$	2-OH-5-Cl	6	0.28	0.12	1.0803	5.40±0.75	-4.53	10.97±0.48 8.14±0.48 0.94±0.10 -3.69±0.10	f
64	Cl	$(CH_3)_3C$	2-CF_3	6	–	0.30	1.6560	4.58±0.68	-4.94	9.22±0.70 -0.35±0.10 4.97±0.10	b
65	H	$(CH_3)_3C$	3,5-OCH_3	5	0.24	0.08	1.0286	4.00±0.54	-3.93	9.73±0.70 1.16±0.10 -3.46±0.10	a

No.	R^1	R^2	R^3	Inhib. [%]	σ_{Ph}	π_{Ph}	log *k*	log *P*	log *S*	pK_a	Ref.
66	H	H	4-Cl	4	0.23	0.16	0.4987	1.84±0.60	-2.72	11.03±0.70 0.13±0.10 -4.49±0.10	g
67	Cl	$(CH_3)_3C$	4-$CH(CH_3)_2$	4	-0.15	0.37	1.7297	5.22±0.65	-5.10	9.96±0.70 0.62±0.50 -4.66±0.10	d
68	Cl	H	3-OCH_3	2	0.12	-0.02	0.6671	2.41±0.64	-3.08	9.45±0.70 -0.17±0.50	a
69	H	$(CH_3)_3C$	2,6-CH_3	2	0.20	0.08	1.1387	3.94±0.52	-3.96	10.67±0.70 1.51±0.50 -3.37±0.10	e
70	Cl	H	2,4-F	1	0.53	0.14	0.8164	1.69±0.73	-3.59	7.81±0.70 -0.05±0.50	d
71	H	H	4-F	0	0.06	0.05	0.4416	*	*	*	b
72	H	H	2,6-Cl	0	1.34	0.32	0.6656	*	*	*	g
73	H	H	2-Cl-5-OH	0	0.79	0.11	0.4527	*	*	*	b
74	Cl	H	2-OH	0	-0.09	-0.08	0.6447	*	*	*	f
75	Cl	H	2-CH_3	0	0.10	0.13	0.7774	*	*	*	e
76	Cl	H	2,6-CH_3	0	0.20	0.20	0.8451	*	*	*	e
77	Cl	H	2,6-Cl	0	1.34	0.32	0.9696	*	*	*	g
78	Cl	H	2-OH-5-Br	0	0.30	0.17	0.8305	*	*	*	a
79	H	$(CH_3)_3C$	2-CH_3	0	0.10	0.13	1.0984	*	*	*	e
80	H	$(CH_3)_3C$	3-Cl	0	0.37	0.14	1.0996	*	*	*	g
81	H	$(CH_3)_3C$	4-Cl	0	0.23	0.16	1.1043	*	*	*	g
82	H	$(CH_3)_3C$	2-OH-5-Br	0	0.30	0.17	1.1070	*	*	*	a
83	H	$(CH_3)_3C$	2-Cl-5-OH	0	0.79	0.11	1.0654	*	*	*	b
84	H	$(CH_3)_3C$	2,6-Cl	0	1.34	0.32	1.2802	*	*	*	g
85	Cl	$(CH_3)_3C$	2-CH_3	0	0.10	0.13	1.4772	*	*	*	e
86	Cl	$(CH_3)_3C$	3-Cl	0	0.37	0.14	1.4896	*	*	*	g
87	Cl	$(CH_3)_3C$	4-CH_3	0	-0.17	0.07	1.3305	*	*	*	b
88	Cl	$(CH_3)_3C$	2-Cl-5-OH	0	0.79	0.11	1.4335	*	*	*	b
89	Cl	$(CH_3)_3C$	2,6-Cl	0	1.34	0.32	1.6631	*	*	*	g
90	Cl	$(CH_3)_3C$	3,4-Cl	0	0.60	0.38	1.7563	*	*	*	a
91	Cl	$(CH_3)_3C$	3,5-OCH_3	0	0.24	0.08	1.3564	*	*	*	a

Table 1. Structures, antimycobacterial evaluation (% of inhibition), logarithms of capacity factors (log *k*), calculated lipophilicity (log *P*), solubility (log *S*) and acid-base properties (pK_a) of compounds **1-91** in comparison with the standard (PZA). Electronic parameters are expressed as Hammett's σ parameter (Ref. Norrington et al., 1975). Compounds are ordered according to their decreasing inhibiting activity (Ref Doležal et al., 2008[(a)], 1999[(b)], 2000[(c)], 2006b[(d)], 2002[(e)], 2010[(f)]; Kutilová, 2007[(g)]). *MIC = 16-50 μg/mL at pH 5.5 (Ref. McDermott & Tompsett, 1954) or MIC = 100 μg/mL at pH 6.0 by the BACTEC method (Ref. Siddiqi, 1992). **ACD values were not calculated for activity 0%.

It can be drawn from Table 1 that growth inhibition of *M. tuberculosis* strain H37Rv at 6.25 μg/mL ranged 0-99%: 0% activity was shown by 21 compounds, 1-49% activity was possessed by 48 compounds and 50-99% activity was shown by 22 compounds, which will be primarily discussed below. According to pyrazine substitution, all compounds in Table 1

can be divided into 4 groups: (*i*) ***series I*** – unsubstituted pyrazine core; (*ii*) ***series II*** – pyrazine substituted by chlorine in position 6 (R^1 = Cl); (*iii*) ***series III*** – pyrazine substituted by *tert*-butyl in position 5 (R^2 = *tert*-Bu); (*iv*) ***series IV*** – pyrazine substituted by chlorine in position 6 and by *tert*-butyl in position 5 (R^1 = Cl and R^2 = *tert*-Bu). When the compounds with the activity above 50% are divided into the individual series, ***series I*** contains 2 compounds with average activity 93%, ***series II*** contains 6 compounds with average activity 69%, ***series III*** contains 7 compounds with average activity 59%, and ***series IV*** contains 7 compounds with average activity 85%. According to this analysis it can be stated that the most favourable is $C_{(5)}$ and $C_{(6)}$ disubstitution of pyrazine R^1 = Cl and R^2 = *tert*-Bu, *i.e.* ***series IV***, followed by unsubstituted pyrazine (***series I***) and $C_{(6)}$ monosubstitution R^1 = Cl (***series II***). The least favourable is $C_{(5)}$ monosubstitution of pyrazine by *tert*-Bu, *i.e.* ***series III***. Particular substituents on benzenes can be ordered according to their efficiency. Benzene substitutions 2,6-Cl, 2-X-5-OH, 2-OH-5-X, 4-X, 3-alkyl, 3-X and 2-CH_3 (X = halogen) appear to be the least advantageous. The most advantageous is substitution 3-CF_3 followed by 4-CH_3, 4-$CH(CH_3)_2$, 3-F and 3,5-CF_3. In all cases this is substitution in *meta* and/or *para* positions, mostly by lipophilic substituents (experimentally determined distributive parameters π_{Ph} = 0.03–0.51 with optimum π_{Ph} = 0.07–0.19). Moieties *meta*-CF_3 and 3-F are electron-withdrawing substituents with σ = 0.43 or σ = 0.34, whereas substituents 4-CH_3 or 4-$CH(CH_3)_2$ with σ = -0.17 or σ = -0.15 are significantly electron-donating.

Substituents with hydrophilic groups on the benzene part of the molecule did not show any inhibiting activity. However, it is worth mentioning that only in ***series IV*** substitutions 4-OH and 2-OH are the most favourable, thus with markedly hydrophilic π_{Ph} = -0.21 or π_{Ph} = -0.08 as well as electron-donating substituents with σ = -0.37 or σ = -0.09. Generally, it can be stated that the optimum lipophilicity value range is log *k* = 0.6–1.5 or log *P* = 1.6–6.8, but as regards antitubercular activity (see Table 1), it can be concluded that lipophilicity is only a secondary parameter, facilitating penetration through highly lipophilic mycobacterial wall. According to Table 1 and log *S* (solubility values) it can be stated that with lower aqueous solubility (lower value of log *S*), antimycobacterial inhibition decreases, except ***series I***. The dissociation constant pK_a is also a very important parameter. According to Table 1 it can be generally stated that higher pK_a (basicity close to PZA) supports higher antitubercular activity.

The selected anilides **1-4** that expressed the highest antitubercular activity are illustrated in Table 2. As mentioned in Section 3, the most effective compounds were tested for their cytotoxicity, and subsequently the Selectivity Index, *i.e.* the ratio of cell cytotoxicity (CC_{50}) to activity (MIC) was obtained. It can be drawn from Table 2 that compound **1** from ***series I*** showed the highest inhibition activity and also minimal toxicity. According to MIC it can be stated that compound **4** seems to be more effective than compound **1**, but unfortunately its determined SI value was not as favourable as in the case of compound **1**. Based on these observations it can be concluded that *N*-[3-(trifluoromethyl)phenyl]pyrazine-2-carboxamide (**1**) can be considered to be a promising agent.

Compounds **92-103** mentioned in Table 3 showed a broad spectrum of antitubercular activity, but these activities are not expressed in concrete numbers, unlike inhibition activities in Table 1, therefore no thorough structure-activity relationships could be

established. Nevertheless, with regard to this fact, it is possible to propose at least general SAR based on the above-discussed observations.

In terms of PZA substitution ***series IV*** is the most advantageous, and the most favourable substitution on the benzene ring is 3-I-4-CH_3. All effective compounds listed in Table 2 possess high lipophilicity; the most active compound **92** has log *k* about 1.9 or log *P* about 6, which enables good penetration to mycobacterial cell. Calculated solubility is relatively low, but under the testing conditions it was probably sufficient, so there was no precipitation during testing.

No.	R^1	R^2	R^3	Inhib. [%]	MIC (μg/mL)	CC_{50} (μg/mL)	SI	Ref.
1	H	H	3-CF_3	**99**	6.25	>62.5	**>10**	a
2	Cl	$(CH_3)_3C$	4-OH	**97**	<12.5	-	-	b
3	Cl	$(CH_3)_3C$	2-OH	**96**	<12.5	-	-	b
4	Cl	$(CH_3)_3C$	3-CF_3	**95**	3.13	>10	>3.2	a

Table 2. Structures, primary antimycobacterial evaluation (% of inhibition), actual minimum inhibitory concentrations (MIC), cytotoxicity (CC_{50}), and selectivity indices (SI) of compounds **1-4** (Ref. Doležal et al., 2008[(a)], 1999[(b)]).

No.	R^1	R^2	R^3	IC_{90} [μg/mL]	σ_{Ph}	π_{Ph}	log *k*	log *P*	log *S*	pK_a
PZA	**see Fig. 1, structure I**			**>20 ***	**–**	**–**	**–**	**-0.71±0.28**	**-0.76**	**13.91±0.50 0.87±0.10**
92	Cl	$(CH_3)_3C$	3-I-4-CH_3	**0.819**	0.18	0.56	1.9783	6.10±0.71	-5.42	9.43±0.70 -0.11±0.50 -4.92±0.10
93	Cl	$(CH_3)_3C$	4-CF_3	**20.703**	0.74	0.23	1.5611	4.39±0.68	-4.87	9.27±0.70 -0.41±0.10
94	H	H	3-I-4-CH_3	**69.099**	0.18	0.56	0.8118	4.12±0.58	-3.56	11.35±0.70 0.10±0.10 -4.53±0.10
95	H	H	4-CF_3	>100	0.74	0.23	0.6220	2.41±0.54	-3.00	11.19±0.70 -0.02±0.10 -4.63±0.10
96	H	H	2-Br-3-CH_3	>100	0.64	0.63	0.8784	3.22±0.57	-3.32	10.98±0.70 0.17±0.10 -4.45±0.10
97	Cl	H	4-CF_3	>100	0.74	0.23	0.8536	2.81±0.67	-3.65	9.20±0.70 -1.39±0.10
98	Cl	H	2-Br-3-CH_3	>100	0.64	0.63	1.2118	3.62±0.70	-3.98	8.98±0.70 -0.65±0.50
99	Cl	H	3-I-4-CH_3	>100	0.18	0.56	1.1529	4.52±0.70	-4.21	9.35±0.70 -0.11±0.50
100	H	$(CH_3)_3C$	4-CF_3	>100	0.74	0.23	1.1606	4.11±0.55	-4.27	9.93±0.70 0.96±0.10

No.	R^1	R^2	R^3	IC_{90} [μg/mL]	σ_{Ph}	π_{Ph}	log *k*	log *P*	log *S*	pK_a
										-3.65±0.10
101	H	$(CH_3)_3C$	3-I-4-CH_3	>100	0.18	0.56	1.5766	5.82±0.59	-4.82	10.09±0.70 1.08±0.10 -3.55±0.10
102	H	$(CH_3)_3C$	2-Br-3-CH_3	>100	0.64	0.63	1.6327	4.92±0.59	-4.60	9.72±0.70 1.15±0.10 -3.47±0.10
103	Cl	$(CH_3)_3C$	2-Br-3-CH_3	>100	0.64	0.63	2.0821	5.20±0.71	-5.19	9.06±0.70 -0.22±0.10 -4.84±0.10

Table 3. Structures, antimycobacterial evaluation (% of inhibition), logarithms of capacity factors (log *k*), calculated lipophilicity (log *P*), solubility (log *S*) and acid-base properties (pK_a) of compounds **92-103** in comparison with the standard (PZA). Electronic parameters are expressed as Hammett's σ parameter (Ref. Norrington et al., 1975). Compounds are ordered according to their decreasing inhibiting activity (Ref. Doležal et al., 2009).
*MIC = 16-50 μg/mL at pH 5.5 (Ref. McDermott & Tompsett, 1954) or MIC = 100 μg/mL at pH 6.0 by the BACTEC method (Ref. Siddiqi, 1992).

According to pK_a these compounds are little less basic than PZA, which results in lower ionizability and higher penetration to the cell. Both types of active anilides have unsubstituted $C'_{(2)}$ position of benzene, which is also very important for easy anilide hydrolysis and release of active POA. It can be assumed that antimycobacterial activity is dependent on balanced lipo/hydrophilic properties and ionizability tending to the PZA value, but also it is strongly influenced by the facility of anilide hydrolysis to acid, which is facilitated by a free *ortho* position of the benzene ring and electron-withdrawing effect of substituents.

5.2 Substituted *N*-(thiazol-2-yl)pyrazine-2-carboxamides

A unique series of ring-substituted *N*-(thiazol-2-yl)pyrazine-2-carboxamides **104-115** is shown in Fig. 9. This series was prepared by the same method as used for substituted *N*-phenylpyrazine-2-carboxamides **1-103**. Their physico-chemical properties and their inhibition of *M. tuberculosis* H37Rv at 6.25 μg/mL are listed in Table 4. It can be concluded that contrary to benzene analogues, *tert*-Bu (***series III***) or Cl+*tert*-Bu (***series IV***) is more advantageous than PZA substituent. The substitution by methyl in position 5 of the 2-aminothiazole ring is unambiguously the most advantageous. The activity of the most active compound **104** is comparable with the activity of compound **18**; but in general the series 5-CH_3 (**104**, **105**, **112**, **113**) possesses lower activity than 4-CH_3 anilides (**6**, **12**, **18**, **87**). 5-Methylthiazolyl pyrazinecarboxamides expressed higher effect than 3-CH_3 (**31**, **36**, **59**) and

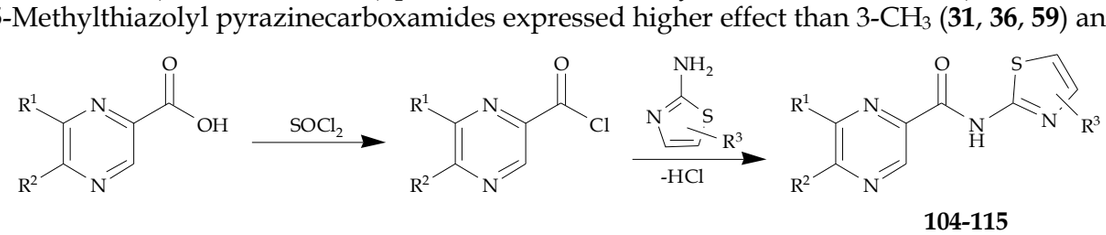


Fig. 9. Synthesis of substituted *N*-(thiazol-2-yl)pyrazine-2-carboxamides **104-115**.

also than 2-CH_3 (**75**, **79**, **85**) anilides. In comparison with anilide analogues, 2-aminothiazoles possess little higher lipophilicity, lower solubility but considerably lower basicity, which in combination with heteroatoms of a thiazole moiety presumably influences the facility of hydrolysis ability and consequently, the resulting activity. Experimentally determined distributive parameters π_{Th} for methylthiazolyl substituents are presented in Table 4.

No.	R^1	R^2	R^3	Inhib. [%]	π_{Th}	log *k*	log *P*	log *S*	pK_a
PZA	**see Fig. 1, structure I**			**	**–**	**–**	**-0.71±0.28**	**-0.76**	**13.91±0.50 0.87±0.10**
104	H	$(CH_3)_3C$	5-CH_3	**65**	0.14	1.0670	4.50±0.28	-4.66	4.35±0.70 1.98±0.10 -0.67±0.10
105	Cl	$(CH_3)_3C$	5-CH_3	**61**	0.14	1.4425	5.23±0.36	-5.29	3.69±0.70 1.77±0.10 -2.04±0.10
106	Cl	$(CH_3)_3C$	4-CH_3	**52**	0.12	1.4174	5.22±0.36	-5.29	3.69±0.70 1.80±0.10 -2.04±0.10
107	H	$(CH_3)_3C$	H	47	0	0.8987	4.20±0.27	-4.44	4.35±0.70 1.50±0.10 -0.68±0.10
108	Cl	$(CH_3)_3C$	H	42	0	1.2282	4.93±0.36	-5.08	3.69±0.70 1.28±0.10 -2.05±0.10
109	H	$(CH_3)_3C$	4-CH_3	35	0.12	1.0446	4.49±0.28	-4.65	4.35±0.70 2.01±0.10 -0.67±0.10
110	Cl	H	H	32	0	0.6064	3.34±0.27	-4.09	3.61±0.70 1.26±0.10 -3.03±0.10
111	Cl	H	4-CH_3	21	0.12	0.7068	3.63±0.28	-4.29	3.62±0.70 1.78±0.10 -3.02±0.10
112	Cl	H	5-CH_3	15	0.14	0.7213	3.64±0.28	-4.29	3.62±0.70 1.74±0.10 -3.02±0.10
113	H	H	5-CH_3	10	0.14	0.6193	2.80±0.27	-3.58	5.61±0.70 1.96±0.10 -1.65±0.10
114	H	H	H	0	0.00	0.5565	*	*	*
115	H	H	4-CH_3	0	0.12	0.6112	*	*	*

Table 4. Structures, antimycobacterial evaluation (% of inhibition), logarithms of capacity factors (log *k*), calculated lipophilicity (log *P*), solubility (log *S*) and acid-base properties (pKa) of substituted *N*-(thiazol-2-yl)pyrazine-2-carboxamides **104-115** in comparison with the standard (PZA). Electronic parameters are expressed as Hammett's σ parameter (Ref. Norrington et al., 1975). Compounds are ordered according to their decreasing inhibiting activity (Ref. Doležal et al., 2006b). *MIC = 16-50 μg/mL at pH 5.5 (Ref. McDermott & Tompsett, 1954) or MIC = 100 μg/mL at pH 6.0 by the BACTEC method (Ref. Siddiqi, 1992). **ACD values were not calculated for activity 0%.

Based on the above-mentioned results and discussion, it can be concluded that for high antitubercular activity $C_{(5)}$ and $C_{(6)}$ disubstitution of the pyrazine ring by lipophilic moieties is advantageous. Substitution of amidic hydrogen by ring-substituted anilines is more advantageous than by heteroaromatics, for example substituted pyrazine-2-carboxylic acid thiazol-2-ylamides showed only medium or moderate activity, and ring-substituted pyrazine-2-carboxylic acid pyridin-2-ylamides (non-mentioned in this chapter) did not express any antimycobacterial activity (Osička, 2003). High antitubercular efficiency requires substitution in *para* or preferentially in *meta* position of benzene, not in *ortho* position. Two combinations of substituents seem to be favoured: (*i*) lipophilic together with electron-withdrawing effect (3-CF_3, 3-F) or (*ii*) expressively hydrophilic together with electron-donating effect (phenolic moiety), $C_{(5)}$ and $C_{(6)}$ disubstituted lipophilic pyrazine being strongly needed for the phenolic type of substituents. The same as PZA, the discussed amides are prodrugs that possess modified absorbency/permeability due to substitution, and after penetration through mycobacterial wall the amides are probably hydrolyzed by intracellular amidase to POA. The sufficient chemical stability of the mentioned compounds in slightly acidic environment used in testing is important for high antitubercular activity as well as subsequent facile hydrolysis by mycobacterial amidase. It seems that electron-withdrawing substituents in *meta* and/or *para* positions provide adequate stability to the amidic bond and simultaneously do not protect this bond against the attack of amidase. No primary physico-chemical parameter that would obviously influence antitubercular inhibitory activity of the discussed substituted pyrazine-2-carboxamides was found. Apart from the balanced combination of secondary parameters (e.g. lipophilicity, solubility, substituent electronic parameters), a high enzymatic hydrolysis rate seems to be fundamental for rapid acidification of mycobacterial cytoplasm (by POA) or inhibition of some other cellular vital enzymatic systems by means of generated reactive intermediates (e.g. aminophenols, fluorinated anilines).

6. Future research

In the recent past, drug discovery efforts shifted towards the drug design based on docking studies. These docking computational techniques allow investigating the possible binding modes of a substrate to a given receptor, enzyme or another binding site and consequently determining and identifying the precise or different mechanism of action of both PZA and its derivatives, e.g. 5-chloropyrazine-2-carboxamide (**IX**) and similar compounds. Therefore the priority is to isolate the enzymes (from *M. tuberculosis* and subsequently from cells of other *Mycobacterial* strains) responsible for metabolization/activation of PZA and other enzymes that can be influenced by POA generation, e.g. FAS I, FAS II, etc. After isolation and determination of 3D structure by X-Ray structural analysis, it will be needed to crystallize enzymes with PZA and other PZA derivatives, determine 3D structures of these complexes and develop 3D-pharmacophore for systematic virtual screening based on this process. Then it will be possible to evaluate all PZA-like derivatives based on their virtual binding simulation and to carry out their evaluation using a large set of distance-based topological indices. In addition, various molecular descriptors can be used.

As most pyrazine derivatives seem to be prodrugs and their activity is strongly dependent on the rate of hydrolysis to POA, further *in vitro* experiments with isolated enzymes should

be focused on determination of the hydrolysis rate. Also compounds of interest will be subject to *in vivo* studies for determination of their efficacy against murine *M. tuberculosis*.

7. Conclusion

PZA is a cornerstone drug of current TB therapy and emerged as an important building block for regimens with promise to shorten TB treatment. PZA is a prodrug which must be activated by the *M. tuberculosis* enzyme pyrazinamidase within the bacterium in order to exert its antitubercular activity. The project was focused on discovery of PZA analogues with PZA-like efficiency characteristics along with improved potency and increased safety. The main task was search for new antimycobacterial pyrazines – structure analogues of PZA. In summary, 115 compounds were synthesized and screened for their antimycobacterial activity in the project. Chemical synthesis was followed by structure confirmation, experimental lipophilicity determination (log *k*) and theoretical lipophilicity calculation (log *P*). Biological evaluation comprised of antimycobacterial activity screening as the main task. This biological part of the project was successfully implemented in co-operation between TAACF, Southern Research Institute, Birmingham, USA, and the Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic, in years 1996—2009. The most active compounds are presented (see Tables 1-4) and discussed. Several very potent compounds (**1-4**, **92**, **93**) were discovered. The compounds relieved into level 2 testing underwent MIC and CC_{50} determination followed by Selectivity Index calculation (SI, ratio of measured CC_{50} to MIC). To be relieved to level 3 (*in vivo* screening) the compound had to exhibit SI>10. Only one of the presented structures, *N*-[3-(trifluoromethyl)phenyl]pyrazine-2-carboxamide (**1**) was relieved to level 3. The *in vivo* screening of compound **1** was not finished yet. The results of this project could be a very good starting point for the advanced drug design and development of new antituberculous agents based on pyrazine.

8. Acknowledgment

Thanks to all our colleagues and numerous graduate and undergraduate students who participated on the synthesis of biologically active pyrazines. The work was partly supported by the Ministry of Health of the Czech Republic (IGA NS 10367-3). Antimycobacterial data were provided in years 1996—2009 by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the U. S. National Institute of Allergy and Infectious Diseases. We take this opportunity to convey our sincere thanks to Dr. Joseph Maddry, the coordinator of the TAACF project. The authors thank Natalia Jampílková for her valuable assistance with the English proofreading of the manuscript.

9. References

ACD/Labs, ver. 12.01 (Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2010)

Adams, J.; Ma, Y.-T.; Stein, R.; Baevsky, M.; Grenier, L. & Plamondon, L. (ProScript, Inc.), (1996, 1998). *Boronic ester and acid compounds*, US 5780454, idem, WO 9613266

Aldrich, C. C.; Bosshoff, H. I. & Remmel R. P. (2010). Antitubercular agents, In: *Burger's Medicinal Chemistry, Drug Discovery and Development*, vol. 7 *Antiinfectives*, Abraham,

D. J. & Rotella, D. P. (Eds.), 7th ed., pp.713–812, Wiley, ISBN 978-0-470-27815-4, New York

Ambrogi, V.; Bloch, K.; Parenti, M. A.; Daturi, S. & Logemann, W. (1972). Synthesis of pyrazine derivatives as potential hypoglycemic agents. *Journal of Pharmaceutical Sciences*, Vol.61, No.9, pp.1483-1489, ISSN 0022-3549

Ambrogi, V.; Cozzi, P.; Sanjust, P.; Bertone, L.; Lovisolo, P. P.; Briaticovangosa, G. & Angelucci, R. (1980). Anti-lipolytic activity of a series of pyrazine-*N*-oxides. *European Journal of Medicinal Chemistry*, Vol.15, No.2, pp.157-163, ISSN 0223-5234

Awwadi, F. F.; Landee, Ch. P.; Turnbull, M. M.; Twamley, B. & Wells, B. M. (2005). Low-dimensional quantum magnetic systems: Synthesis, structure and magnetic behavior of (2,5-dimethylpyrazine)copper(ii) chloride and synthesis and magnetic behavior of bis(2,6-dimethylpyrazine)copper(ii) chloride. *Polyhedron*, Vol.24, No.16-17, pp.2153-2159, ISSN 0277-5387

Bergmann K. E.; Cynamon M. H. & Welch J. T. (1996). Quantitative structure-activity relationships for the *in vitro* antimycobacterial activity of pyrazinoic acid esters. *Journal of Medicinal Chemistry*, Vol.39, No.17, pp.3394-3400, ISSN 0022-2623

Bird, C. W. (1992). Heteroaromaticity, 5, a unified aromaticity index. *Tetrahedron*, Vol.48, No.2, pp.335-340, ISSN 0040-4020

Boshoff, H. I.; Mizrahi, V. & Barry, C. E. 3rd (2002). Effects of pyrazinamide on fatty acid synthesis by whole mycobacterial cells and purified fatty acid synthase I. *Journal of Bacteriology*, Vol.184, No.8, pp.2167-2172, ISSN 1098-5530

Brennan, P. J. & Nikaido, H. (1995). The envelope of mycobacteria. *Annual Review of Biochemistry*, Vol.64, pp.29-63, ISSN 1545-4509

Brown, D. J. (2002). *The Pyrazines, Supplement I. The Chemistry of Heterocyclic Compounds.* p.576, John Wiley & Sons, ISBN 9780471403821, New York

Camus, J.-C., Pryor, M. J.; Medigue, C. & Cole, S. T. (2002). Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv. *Microbiology*, Vol.148, No.10, pp.2967-2973, ISSN 1350-0872

Chorine, V. M. (1945). Médecine expérimentale – Action de l'amide nicotinique sur les bacilles du genre mycobacterium. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, Vol.220, No.4, pp.150-156, ISSN 0001-4036

Chung, W. J.; Kornilov, A.; Brodsky, B. H.; Higgins, M.; Sanchez, T.; Heifets, L. B.; Cynamon, M. & Welch, J. (2008). Inhibition of *M. tuberculosis in vitro* in monocytes and in mice by aminomethylene pyrazinamide analogs. *Tuberculosis*, Vol.88, No.5, pp.410-419, ISSN 1027-3719

Clapper, M. L. (1998). Chemopreventive activity of oltipraz. *Pharmacology and Therapy*, Vol.78, No.1, pp.17-27, ISSN 0163-7258

Coe, J. W.; Brooks, P. R.; Vetelino, M. G.; Wirtz, M. C.; Arnold, E. P.; Huang, J.; Sands, S. B.; Davis, T. I.; Lebel, L. A.; Fox, C. B.; Shrikhande, A.; Heym, J. H.; Schaeffer, E.; Rollema, H.; Lu, Y.; Mansbach, R. S.; Chambers, L. K.; Rovetti, Ch. C.; Schulz, D. W.; Tingley, F. D. 3rd & O'Neill, B. T. (2005). Varenicline: An α4β2 nicotinic receptor partial agonist for smoking cessation. *Journal of Medicinal Chemistry*, Vol.48, No.10, pp.3474-3477, ISSN 0022-2623

Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry C. E. 3rd; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin, K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborne, J.; Quail, M. A.; Rajandream, M.-A.; Rogers, J.; Rutter, S.; Seeger, K.; Skelton, J.; Squares, R.; Squares, S.; Sulston, J. E.; Taylor, K.;

Whitechead, S. & Barrell B. G. (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature*, Vol.393, No.6685, pp.537-544, ISSN 0028-0836

Cotrel, C.; Jeanmart, C. & Messer, M. N. (Rhone-Poulenc, Aventis Pharma SA), (1973, 1975). *Pyrrolo(3,4-b)pyrazine derivatives*, DE 2300491, idem, US 3862149

Collins, L. A. & Franzblau, S. G. (1997). Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrobial Agents and Chemotherapy*, Vol.41, No.5, pp.1004–1009, ISSN 0066-4804

Cragoe, E. J. Jr. (Merck & Co.), (1964, 1967). *(3-Amino-5,6-disubstituted-pyrazinoyl)guanidines*, BE 639386, idem, US 3313813

Cynamon, M. H.; Klemens, S. P.; Chou T. S.; Gimi, R. H. & Welch, J. T. (1992). Antimycobacterial activity of a series of pyrazinoic acid-esters. *Journal of Medicinal Chemistry*, Vol.35, No.7, pp.1212-1215, ISSN 0022-2623

Cynamon, M. H.; Gimi, R.; Gyenes, F.; Sharpe, C. A.; Bergmann, K. E.; Han, H. J.; Gregor, L. B.; Rapolu, R.; Luciano, G. & Welch, J. T. (1995). Pyrazinoic acid esters with broad spectrum *in vitro* antimycobacterial activity. *Journal of Medicinal Chemistry*, Vol.38, No.20, pp.3902–3907, ISSN 0022-2623

Cynamon, M. H.; Speirs, R. J. & Welch, J. T. (1998). *In vitro* antimycobacterial activity of 5-chloropyrazinamide. *Antimicrobial Agents and Chemotherapy*, Vol.42, No.2, pp.462-463, ISSN 0066-4804

Dalmer, O. & Eugen, W. (E. Merck), (1936). *Pyrazine derivatives*, DE 632257

Dessau, F. I.; Burger, F. J.; Yeager, R. L. & Kulish, M. (1952). A method for the determination of *in vitro* sensitivity of tubercle bacilli to pyrazinamide (aldinamide). *American Review of Tuberculosis*, Vol.65, No.5, pp.635–636, ISSN 0096-0381

Dlabal, K.; Palát, K.; Lyčka, A. & Odlerová, Ž. (1990). Synthesis and proton and carbon-13 NMR spectra of sulfur derivatives of pyrazine derived from amidation product of 2-chloropyrazine and 6-chloro-2-pyrazinecarbonitrile. Tuberculostatic activity. *Collection of Czechoslovak Chemical Communications*, Vol.55, No.10, pp.2493-2501, ISSN 0010-0765

Doležal, M.; Jampílek, J.; Osička, Z.; Kuneš, J.; Buchta, V. & Víchová, P. (2003). Substituted 5-aroylpyrazine-2-carboxylic acid derivatives: Synthesis and biological activity. *Farmaco*, Vol.58, No.11, pp.1105-1111, ISSN 0014-827X

Doležal, M. (2006a). Biological active pyrazines of natural and synthetic origin. *Chemické Listy*, Vol.100, No.11, pp.959-966, ISSN 0009-2770

Doležal, M.; Palek, L.; Vinšová, J.; Buchta, V.; Jampílek, J. & Kráľová, K. (2006b). Substituted pyrazinecarboxamides: Synthesis and biological evaluation. *Molecules*, Vol.11, No.4, pp.242-256, ISSN 1420-3049

Doležal, M.; Tůmová, L.; Kešetovičová, D.; Tůma, J. & Kráľová, K. (2007). Substituted *N*-phenylpyrazine-2-carboxamides, their synthesis and evaluation as herbicides and abiotic elicitors. *Molecules*, Vol.12, No.12, pp.2589-2598, ISSN 1420-3049

Doležal, M.; Čmedlová, P.; Palek, L.; Vinšová, J.; Kuneš, J.; Buchta, V.; Jampílek, J. & Kráľová, K. (2008). Synthesis and antimycobacterial evaluation of substituted pyrazinecarboxamides. *European Journal of Medicinal Chemistry*, Vol.43, No.5, pp.1105-1113, ISSN 0223-5234

Doležal, M.; Zitko, J.; Kešetovičová, D.; Kuneš, J. & Svobodová, M. (2009). Substituted *N*-phenylpyrazine-2-carboxamides: Synthesis and antimycobacterial evaluation. *Molecules*, Vol.14, No.10, pp.4180–4189, ISSN 1420-3049

Doležal, M.; Zitko, J.; Osička, Z.; Kuneš, J.; Buchta, V.; Vejsová, M.; Dohnal, J.; Jampílek, J. & Kráľová K. (2010). Synthesis, antimycobacterial, antifungal and photosynthesis-inhibiting activity of chlorinated *N*-phenylpyrazine-2-carboxamides. *Molecules*, Vol.15, No.12, pp.8567–8581, ISSN 1420-3049

Doležal, M. & Kráľová K. (2011). Synthesis and evaluation of pyrazine derivatives with herbicidal activity, In: *Herbicides, Theory and Applications*, Soloneski, S. & Larramendy, M. L. (Eds.), pp.581-610, InTech, ISBN 978-953-307-975-2, Rijeka, Retrieved from <http://www.intechopen.com/articles/show/title/synthesis-and-evaluation-of-pyrazine-derivatives-with-herbicidal-activity>

Durán, R.; Zubía, E.; Ortega, M. J.; Naranjo, S. & Salvá, J. (1999). Novel alkaloids from the red ascidian *Botryllus leachi*. *Tetrahedron*, Vol.55, No.46, pp.13225-13232, ISSN 0040-4020

Edelman M. J.; Meyers, F. J.; Grennan, T.; Lauder, J. & Doroshow, J. (1998). Phase II trial of pyrazine diazohydroxide in androgen-independent prostate cancer. *Investigational New Drugs*, Vol.16, No.2, pp.179-182, ISSN 0167-6997

Felder, E. & Tiepolo, U. (Bracco), (1962). *Preparation from pyrazinamide, morpholine, and formalin*, DE 1129492

Fernandes, J. P. S.; Pasqualoto, K. F. M.; Felli, V. M. A.; Ferreira, E. I. & Brandt, C. A. (2010). QSAR Modeling of a set of pyrazinoate esters as antituberculosis prodrugs. *Archiv der Pharmazie – Chemistry in Life Sciences*, Vol.343, No.2, pp.91-97, ISSN 0341-2407

Foreman, M. M.; Hanania, T.; Stratton, S. C.; Wilcox, K. S.; White, H. S.; Stables, J.P. & Eller, M. (2008). *In vivo* pharmacological effects of JZP-4, a novel anticonvulsant, in models for anticonvulsant, antimania and antidepressant activity. *Pharmacology Biochemistry and Behavior*, Vol.89, No.4, pp.523-534, ISSN 0091-3057

France, C. P.; Winger, G.; Medzihradsky, F.; Seggel, M. R.; Rice, K. C. & Woods, J. H. (1991). Mirfentanil: Pharmacological profile of a novel fentanyl derivative with opioid and nonopioid effects. *Journal of Pharmacology and Experimental Therapeutics*, Vol.258, No.2, pp.502-510, ISSN 0022-3565

Furuta, Y.; Takahashi, K.; Shirakib, K.; Sakamoto, K.; Smee, D. F.; Barnard, D. L.; Gowen, B. B.; Julander, J. G. & Morrey, J. D. (2009). T-705 (favipiravir) and related compounds: Novel broad-spectrum inhibitors of RNA viral infections. *Antiviral Research*, Vol.82, No.3, pp.95-102, ISSN 0166-3542

Global Tuberculosis Control: *WHO Report 2010* (2010). pp.1–42, WHO Press, ISBN 978-92-4-156406-9, Geneva, [cited 2011 July22], Available form: <http://whqlibdoc.who.int/publications/2010/9789241564069_eng.pdf>

Grosset, J. (1978). The sterilizing value of rifampicin and pyrazinamide in experimental short-course chemotherapy. *Bulletin of the International Union against Tuberculosis*, Vol.53, No.1, pp.5-12, ISSN 1011-789X

Hall, S. A. & Spoerri, P. E. (1940). Syntheses in the pyrazine series. II. Preparation and properties of aminopyrazine. *Journal of American Chemical Society*, Vol.62, No.3, pp.664-665, ISSN 0002-7863

Heifets, L.; Iseman, M. D.; Crowle, A. J. & Lindholm-Levy, P. J. (1986). Pyrazinamide is not active *in vitro* against *Mycobacterium avium* complex. *American Review of Respiratory Disease*, Vol.134, No.6, pp.1287-1288, ISSN 0003-0805

Heifets, L. & Lindholm-Levy, P. (1992). Pyrazinamide sterilizing activity *in vitro* against semidormant *Mycobacterium tuberculosis* bacterial populations. *American Review of Respiratory Disease*, Vol.145, No.5, pp.1223–1225, ISSN 0003-0805

Hurdle, J. G.; O'Neill, A. J.; Chopra, I. & Lee, R. E. (2011). Targeting bacterial membrane function: An underexploited mechanism for treating persistent infections. *Nature Reviews Microbiology*, Vol.9, No.1, pp.62-75, ISSN 1740-1526

Jampílek, J.; Doležal, M. & Buchta, V. (2007). Antimicrobial evaluation of some arylsulfanylpyrazinecarboxylic acid derivatives. *Medicinal Chemistry*, Vol.3, No.3, pp.277-280, ISSN 1573-4064

Joule, J. A. & Mills, K. (2010). *Heterocyclic Chemistry*, pp. 249-288, Wiley-Blackwell, ISBN 9781405133005, Chichester

Kikugawa, K. (2004). Organochemical studies on the formation and the reduction of mutagenic and/or carcinogenic and the DNA damaging components in foods. *Environmental Mutagen Research*, Vol.26, No.1, pp.23-31, ISSN 0910-0865

Kim, N. D.; Kwak, M. K. & Kim, S. G. (1997). Inhibition of cytochrome P450 2E1 expression by 2-(allylthio)pyrazine, a potential chemoprotective agent: Hepatoprotective effects. *Biochemical Pharmacology*, Vol.53, No.3, pp.261-269, ISSN 0006-2952

Kolattukudy, P. E.; Fernandes, N. D.; Azad, A. K.; Fitzmaurice, A. M. & Sirakova, T. D. (1997). Biochemistry and molecular genetics of cell-wall lipid biosynthesis in mycobacteria. *Molecular Microbiology*, Vol.24, No.2, pp.263–270, ISSN 0950-382X

Konno, K.; Feldmann, F. M. & McDermott, W. (1967). Pyrazinamide susceptibility and amidase activity of tubercle bacilli. *American Review of Respiratory Disease*, Vol.95, No.3, pp.461-469, ISSN 0003-0805

Kushner, S.; Dalalian, H.; Sanjurjo, J. L.; Bach, F. L. Jr.; Safir, S. R.; Smith, V. K. Jr. & Williams, J. H. (1952). Experimental chemotherapy of tuberculosis. 2. The synthesis of pyrazinamides and related compounds. *Journal of American Chemical Society*, Vol.74, No.14, pp.3617–3621, ISSN 0002-7863

Kutilová, H. (2003). *Pyrazine Derivatives as Potential Drugs I*, Diploma Thesis, Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Lacroix, C.; Hoang, T. P.; Nouveau, J.; Guyonnaud, C.; Laine, G.; Duwoos, H. & Lafont, O. (1989). Pharmacokinetics of pyrazinamide and its metabolites in healthy subjects. *European Journal of Clinical Pharmacology*, Vol.36, No.4, pp.395-400, ISSN 0031-6970

Liu, J.; Barry, C. E. 3rd & Nikaido, H. (1999). Cell wall: Physical structure and permeability, In: *Mycobacteria: Molecular Biology and Virulence*, Ratledge, C. & Dale, J. (Eds.), pp.220-239, Blackwell Science, ISBN 978-0632053049, London

Liu, J.; Barry, C. E. 3rd; Besra, G. S. & Nikaido, H. (1996). Mycolic acid structure determines the fluidity of the bacterial cell wall. *Journal of Biological Chemistry*, Vol.271, No.47, pp.29545-29551, ISSN 0021-9258

MacDonald, J. C. (1965). Biosynthesis of pulcherriminic acid. *Biochemical Journal*, Vol.96, No.2, pp.533-538, ISSN 0264-6021

MacWilliam, I. C. (1959). A survey of the antibiotic powers of yeasts. *Journal of General Microbiology*, Vol.21, No.2, pp.410-414, ISSN 1350-0872

Maga, J. A. (1992). Pyrazine update. *Food Reviews International*, Vol.8, No.4, pp.479-558, ISSN 8755-9129

Malone, L.; Schurr, A.; Lindh, H.; McKenzie, D.; Kiser, J. S. & Williams, J. H. (1952). The effect of pyrazinamide (aldinamide) on experimental tuberculosis in mice. *American Review of Tuberculosis*, Vol.65, No.5, pp.511–518, ISSN 0096-0381

Manabe, Y. C.; Dannenberg, A. M. & Bishai, W. R. (2000). What we can learn from the *Mycobacterium tuberculosis* genome sequencing projects. *International Journal of Tuberculosis and Lung Diseases*, Vol.4, No.2, pp.518-523, ISSN 1027-3719

McCullough, K. J. (1989). Pyrazines and related ring structures. In: *Rodd's Chemistry of Carbon Compounds*, Coffey, S. & Ansell, M. F. (Eds.), pp.294-296, Elsevier Science, ISBN 0444421831, New York

McDermott, W. & Tompsett, R. (1954). Activation of pyrazinamide and nicotinamide in acidic environment *in vitro*. *American Review of Tuberculosis*, Vol.70, No.4, pp.748-754, ISSN 0096-0381

McKenzie, D.; Malone, L.; Kushner, S.; Oleson, J. J. & Subbarow, Y. (1949). The effect of nicotinic acid amide on experimental tuberculosis of white mice. *Journal of Laboratory and Clinical Medicine*, Vol.33, No.10, pp.1249-1253, ISSN 0022-2143

Mdluli, K.; Slayden, R. A.; Zhu, Y. Q.; Ramaswamy, S.; Pan, X.; Mead, D.; Crane, D. D.; Musser, J. M. & Barry, C. E. 3rd (1998). Inhibition of a *Mycobacterium tuberculosis* β-ketoacyl ACP synthase by isoniazid. *Science*, Vol.280, No.5369, pp.1607–1610, ISSN 0036-8075

Micetich, R. G. & MacDonald, J. C. (1965). Biosynthesis of neoaspergillic acid and neohydroxyaspergillic acids. *Journal of Biological Chemistry*, Vol.240, No.4, pp.1692-1695, ISSN 0021-9258

Mitchison, D. A. (1985). The action of antituberculosis drugs in short-course chemotherapy. *Tubercle*, Vol.66, No.3, pp.219-225, ISSN 0041-3879

Müller, R. & Rappert, S. (2010). Pyrazines: Occurrence, formation and biodegradation. *Applied Microbiology and Biotechnology*, Vol.85, No.5, pp.1315-1320, ISSN 0175-7598

Ngo, S. C.; Zimhony, O.; Chung, W. J.; Sayahi, H.; Jacobs, W. R. Jr. & Welch, J. T. (2007). Inhibition of isolated *Mycobacterium tuberculosis* fatty acid synthase I by pyrazinamide analogs. *Antimicrobial Agents and Chemotherapy*, Vol.51, No.7, pp.2430-2435, ISSN 0066-4804

Norrington, F. E.; Hyde, R. M.; Williams, S. G. & Wootton, R. (1975). Physicochemical-activity relations in practice. 1. Rational and self-consistent data bank. *Journal of Medicinal Chemistry*, Vol.18, No.6, pp.604-607, ISSN 0022-2623

Osička, Z. (2003). *Pyrazine Derivatives as Potential Drugs II*, Diploma Thesis, Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Palek, L.; Dvořák, J.; Svobodová, M.; Buchta, V.; Jampílek, J. & Doležal, M. (2008). Synthesis, antimycobacterial and antifungal evaluation of 3-arylaminopyrazine-2,5-dicarbonitriles. *Archiv der Pharmazie – Chemistry in Life Sciences*, Vol.341, No.1, pp.61-65, ISSN 0365-6233

Rajini, R. S.; Aparna, P.; Sasikala, Ch. & Ramana, Ch. V. (2011). Microbial metabolism of pyrazines. *Critical Reviews in Microbiology*, Vol.37, No.2, pp.99-112, ISSN 1040-841X

Raman, K.; Rajagopalan, P. & Chandra, N. (2005). Flux balance analysis of mycolic acid pathway: Targets for anti-tubercular drugs. *PLoS Computational Biology*, Vol.1, No.5, pp. 349-358, ISSN 1553-734X

Roblin, R. O. Jr.; Williams, J. H.; Winnek, P. S. & English, J. P. (1940). Chemotherapy: II. Some sulfanilamide heterocycles. *Journal of American Chemical Society*, Vol.62, No.8, pp.2002-2005, ISSN 0002-7863

Sak-Bosnar, M. &. Kovar, K. (2005). Acid–base characterization of 5-hydroxypyrazine-2-carboxylic acid and the role of ionic equilibria in the optimization of some process conditions for its biocatalytic production. *Analytical and Bioanalytical Chemistry*, Vol.383, No.3, pp.539–545, ISSN 1618-2642

Scorpio, A. & Zhang, Y. (1996). Mutations in PncA, a gene encoding pyrazinamidase/ nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nature Medicine*, 1996, Vol.2, No.6, pp.662-667, ISSN 1078-8956

Siddiqi, S. H. (1992). Antimicrobial susceptibility testing: Radiometric (BACTEC) tests for slowly growing mycobacteria, In: *Clinical Microbiology Procedure Handbook*, Isenberg, H. D. (Ed.), pp.14-25, ASM Press, ISBN 978-1-55581-029-0, Washington D.C.

Simões, M. F.; Valente, E.; Gómez J. R. M.; Anes, E. & Constantino, L. (2009). Lipophilic pyrazinoic acid amide and ester prodrugs stability, activation and activity against *M. tuberculosis*. *European Journal of Pharmaceutical Sciences*, Vol.37, No.3-4, pp.257-263, ISSN 0928-0987

Solotorovsky, M.; Gregory, F. J.; Ironson, E. J.; Bugie, E. J.; O'Neill, R. C. & Pfister, K. (1952). Pyrazinoic acid amide; an agent active against experimental murine tuberculosis. *Proceedings of the Society for Experimental Biology and Medicine*, Vol.79, No.4, pp.563-565, ISSN 0037-9727

Speirs, R. J.; Welch, J. T. & Cynamon, M. H. (1995). Activity of *n*-propyl pyrazinoate against pyrazinamide-resistant *Mycobacterium tuberculosis*: Investigations into mechanism of action of and mechanism of resistance to pyrazinamide. *Antimicrobial Agents and Chemotherapy*, Vol.39, No.6, pp.1269-1271, ISSN 0066-4804

Steele, M. A. & Des Prez, R. M. (1988). The role of pyrazinamide in tuberculosis chemotherapy. *Chest*, Vol.94, No.4 , pp.845-850, ISSN 0012-3692

TAACF [homepage on the Internet] Tuberculosis Antimicrobial Acquisition and Coordinating Facility (2010). [cited 2011 July22]. Available from: <http://www.taacf.org/>

Tarnok, I.; Pechmann, H.; Krallmann Wenzel, U.; Rohrscheidt, E. & Tarnok, Z. (1979). Nicotinamidase and the so-called pyrazinamidase in mycobacteria; the simultaneous occurrence of both activities. *Zentralblatt für Bakteriologie*, Vol.244, No.2-3, pp.302–308, ISSN 0174-3031

The Stop TB Partnership [homepage on the Internet]. World Health Organization [cited 2011 July22]. Available from: <http://www.stoptb.org/assets/documents/global/plan/SSP_DRUGWG.pdf>

Thompson, E. M. & Rees, J. F. (1994). Origins of the luciferins, the ecology of bioluminescence in marine fishes, In: *Biochemistry and Molecular Biology of Fishes*, Hochachka, P. W. & Mommsen, T. P. (Eds.), pp.435–466, Elsevier, ISBN 978-0-444-51833-0, Amsterdam/New York

Thornber, C. W. (1979). Isosterism and molecular modification in drug design. *Chemical Society Reviews*, Vol.8, No.4 , pp.563-580, ISSN 0306-0012

Topliss, J. G. & Costello, R. J. (1972). Chance correlations in structure–activity studies using multiple regression analysis. *Journal of Medicinal Chemistry*, Vol.15, No.10, pp.1066-1068, ISSN 0022-2623

Vargas, F.; Rivas, C.; Diaz, Y. & Fuentes, A. (2003). Photodegradation pathways and the *in vitro* phototoxicity of pyrazinamide, a phototoxic antitubercular drug. *Journal of Photochemistry and Photobiology B – Biology*, Vol.72, No.1-3, pp.87-94, ISSN 1011-1344

Velayati, A. A.; Masjedi, M. R.; Farnia, P.; Tabarsi, P.; Ghanavi, J.; Ziazarifi, A. H. & Hoffner, S. E. (2009). Emergence of new forms of totally drug-resistant tuberculosis bacilli: Super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. *Chest*, Vol.136, No.2, pp.420–425, ISSN 0012-3692

Vinšová, J.; Imramovský, A.; Jampílek, J.; Monreal-Férriz, J.; Doležal, M. (2008). Recent advances on isoniazide derivatives. *Anti-Infective Agents in Medicinal Chemistry*, Vol.7, No.1, pp.12-31, ISSN 1871-5214

Vinšová, J. & Krátký, M. (2010). Tuberculosis – The development of new MDR-TB drugs, In: *Drug-Resistant Tuberculosis, Causes, Diagnosis and Treatments*, Nguy, S. & K'ung, Z. (Eds.), pp.59-141, Nova Science Publishers, ISBN 978-1-60876-055-8, New York

Wade, M. M. & Zhang, Y. (2004). Mechanism of drug resistance in *Mycobacterium tuberculosis*. *Frontiers in Bioscience*, Vol.9, No.1, pp.975-994, ISSN 1093-9946

Wade, M. M. & Zhang, Y. (2006). Effects of weak acids, UV and proton motive inhibitors on pyrazinamide activity against *Mycobacterium tuberculosis in vitro*. *Journal of Antimicrobial Chemotherapy*, Vol.58, No.5, pp.936-941, ISSN 0305-7453

Wagner, R.; Czerny, M.; Bielohradsky, J. & Grosch, W. (1999). Structure-odour-activity relationships of alkylpyrazines. *Zeitschrift für Lebensmitteluntersuchung und - Forschung A*, Vol.208, No.5-6, pp.308-316, ISSN 1431-4630

White, E. C. & Hill, J. H. (1943). Studies on antibacterial products formed by molds: I. Aspergillic acid, a product of a strain of *Aspergillus flavus*. *Journal of Bacteriology*, Vol.45, No.5, pp.433-443, ISSN 0021-9193

Wieser, M.; Heinzmann, K. & Kiener, A. (1997). Bioconversion of 2-cyanopyrazine to 5-hydroxypyrazine-2-carboxylic acid with *Agrobacterium* sp. DSM 6336. *Applied Microbiology and Biotechnology*, Vol.48, No.2, pp.174–176, ISSN 0175-7598

Woolfson, A. & Rothschild, M. (1990). Speculating about pyrazines. *Proceedings of the Royal Society of London Series B - Biological Sciences*, Vol.242, No.1304, pp.113-119, ISSN 0962-8452

Yamaguchi, T. (2007). The relationship between the chemical structures of dihydropyrazine derivatives and DNA strand-breakage activity. *Chemical Pharmaceutical Bulletin*, Vol.55, No.4, pp.532-536, ISSN 0009-2363

Yamamoto, S.; Toida, I.; Watanabe, N. & Ura, T. (1995). *In vitro* antimycobacterial activities of pyrazinamide analogs. *Antimicrobial Agents and Chemotherapy*, Vol.39, No.9, pp.2088-2091, ISSN 0066-4804

Yeager, R. L.; Munroe, W. G. C. & Dessau, F. I. (1952). Pyrazinamide (aldinamide) in the treatment of pulmonary tuberculosis. *American Review of Tuberculosis*, Vol.65, No.5, pp.523–545, ISSN 0096-0381

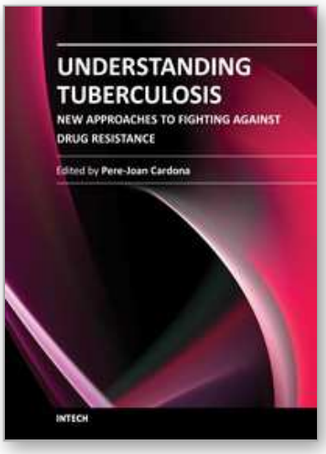
Zhang, Y. & Mitchison, D. A. (2003). The curious characteristics of pyrazinamide: A review. *International Journal of Tuberculosis and Lung Diseases*, Vo.7, No.1, pp.6-21, ISSN 1027-3719

Zhang, Y.; Scorpio, A.; Nikaido, H. & Sun, Z. (1999). Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of *M. tuberculosis* to pyrazinamide. *Journal of Bacteriology*, Vol.181, No.7, pp.2044–2049, ISSN 0021-9193

Zhang, Z.; Wei, T.; Hou, J.; Li, G.; Yu, S. & Xin, W. (2003). Tetramethylpyrazine scavenges superoxide anion and decreases nitric oxide production in human polymorphonuclear leukocytes. *Life Science*, Vol.72, No.22, pp.2465-2472, ISSN 0024-3205

Zierski, M. & Bek, E. (1980). Side effects of drug regimens used in short-course chemotherapy for pulmonary tuberculosis. *Tubercle*, Vol.61, No.1, pp.41-49, ISSN 0041-3879

Zitko, J.; Doležal, M.; Svobodová, M.; Vejsová, M.; Kuneš, J.; Kučera, R. & Jílek, P. (2011). Synthesis and antimycobacterial properties of *N*-substituted 6-amino-5-cyanopyrazine-2-carboxamides. *Bioorganic and Medicinal Chemistry*, Vol.19, No.4, pp.1471-1476, ISSN 0968-0896



Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance

Edited by Dr. Pere-Joan Cardona

ISBN 978-953-307-948-6
Hard cover, 376 pages
Publisher InTech
Published online 15, February, 2012
Published in print edition February, 2012

In 1957, a Streptomyces strain, the ME/83 (S.mediterranei), was isolated in the Lepetit Research Laboratories from a soil sample collected at a pine arboretum near Saint Raphael, France. This drug was the base for the chemotherapy with Streptomicine. The euphoria generated by the success of this regimen lead to the idea that TB eradication would be possible by the year 2000. Thus, any further drug development against TB was stopped. Unfortunately, the lack of an accurate administration of these drugs originated the irruption of the drug resistance in Mycobacterium tuberculosis. Once the global emergency was declared in 1993, seeking out new drugs became urgent. In this book, diverse authors focus on the development and the activity of the new drug families.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Martin Doležal, Jan Zitko and Josef Jampílek (2012). Pyrazinecarboxylic Acid Derivatives with Antimycobacterial Activity, Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance, Dr. Pere-Joan Cardona (Ed.), ISBN: 978-953-307-948-6, InTech, Available from: http://www.intechopen.com/books/understanding-tuberculosis-new-approaches-to-fighting-against-drug-resistance/pyrazinecarboxylic-acid-derivatives-with-antimycobacterial-activity

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.