REVIEW ARTICLE

Focus on perchlozone, an anti-tuberculosis drug from the Russian Federation

Pohľad na perchlozón, antituberkulotikum z Ruskej federácie

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Received September 23, 2020 / Accepted October 7, 2020

Summary

The prevalence of multidrug-resistant tuberculosis (MDR--TB) and extensively drug-resistant tuberculosis (XDR--TB) has been increasing at an alarming rate worldwide. Today's "Fight against Tuberculosis" programmes in the Russian Federation are subsidized by state and regional governments as well as health authorities. Each region has its own specific characteristics and needs specific interventions. Although some novel anti-tuberculosis (anti-TB) drugs (**bedaquiline**, **delamanid**) were approved by relevant authorities, and some promising compounds, especially those of oxazolidinones, are in various phases of clinical trials worldwide, the finding of effective, safe, pharmacokinetically favorable, economically and logistically accessible anti-TB agents still remains a serious challenge for medical and pharmaceutical sciences. **Perchlozone**, a compound containing a thiosemicarbazone scaffold, was approved in the Russian Federation in 2012 for the treatment (alone or as the active component of complex treatment regimens) of HIV-1 negative as well as HIV-1 positive patients suffering from MDR-TB or XDR-TB. Mechanism of anti-TB action of **perchlozone** might be similar to that of **thiacetazone**, which belongs into the same chemical class. **Perchlozone** has to be probably activated into reactive species by a mycobacterially encoded monoxygenase (EthA). The activated forms might act in multiple ways, including inhibition of mycobacterial cell wall synthesis due to interfence with a dehydration step of the type II fatty acid synthase pathway or sensitization of the *Mycobacterium tuberculosis* cell to oxidative stress. Favorable toxicological properties of **perchlozone** and its tolerability by the human organism were confirmed within revevant preclinical and clinical studies. However, recent preliminary investigations *in vivo* (animal models) could indicate genotoxicity after subacute inhalation of the drug. Regarding this issue, further development of more convenient nano- or microparticle-based formulations of **perchlozone** potentially improving targeted delivering and efficiency as well as decreasing (eliminating) its eventual toxicity might be taken into strong consideration.

Key words: perchlozone • *Mycobacterium tuberculosis* • resistance • MDR-TB • XDR-TB • HIV-1 co-infection

Súhrn

Prevalencia multirezistentnej tuberkulózy (multidrug-resistant tuberculosis – MDR-TB) a extenzívne rezistentnej tuberkulózy (extensively drug-resistant tuberculosis – XDR-TB) sa vo svete alarmujúcim tempom zvyšuje. Aktuálne programy „Boja proti tuberkulóze" sú v Ruskej federácii podporované štátnou vládou, regionálnymi vládami a tiež zdravotníckymi inštitúciami. Každý región tento krajiny má však vlastné špecifiká a vyžaduje si špecifické intervencie. Napriek tomu, že niektoré nové antituberkuloticky (anti-TB) pôsobiace liečivá (**bedachilín**, **delamanid**) boli už relevantnými inštitúciami schválené a iné sľubné zlúčeniny, najmä zo skupiny oxazolidinónov, sú v rôznych fázach klinických hodnotení prebiehajúcich vo svete, nájdenie efektívnych, bezpečných, farmakokineticky výhodných, ekonomicky a logisticky dostupných anti-TB-liečiv stále zostáva pre medicínske a farmaceutické vedy veľkou výzvou. Tiosemikarbazónové liečivo **perchlozón** bolo v Ruskej federácii schválené v roku 2012 pre liečbu (samostatne, alebo ako aktívna zložka komplexných liečebných režimov) HIV-1-negatívnych a tiež HIV--1-pozitívnych pacientov, ktorí sú postihnutí MDR-TB alebo XDR-TB. Mechanizmus anti-TB-účinku **perchlozónu** by mohol byť podobný tomu, akým pôsobí **tiacetazón**, ktorý patrí do identickej chemickej skupiny. **Perchlozóm** musí byť pravdepodobne aktivovaný mykobakteriálne kódovanou oxygenázou (EthA) na reaktívne entity. Tieto aktívne formy by mohli pôsobiť viacerými mechanizma-

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mi, vrátane inhibície syntézy bunkovej steny mykobaktérií (kvôli interferencii s procesom dehydratácie syntázy mastných kyselín typu II) alebo senzitizácie bunky *Mycobacterium tuberculosis* voči oxidačnému stresu. V rámci relevantných predklinických a klinických štúdií **perchlozónu** boli potvrdené jeho výhodné farmakokinetické vlastnosti a tiež tolerovateľnosť ľudským organizmom. Aktuálne predbežné zistenia *in vivo* (animálne modely) by však mohli indikovať genotoxicitu po subakútnej inhalácii tohto liečiva. Z uvedeného dôvodu je veľmi žiaduce uvažovať o ďalšom vývoji výhodnejších spôsobov podania **perchlozónu**, ktoré sú založené na nano- a mikročasticových systémoch. Tieto inovatívne alternatívy by potenciálne zlepšili cielené dodanie liečiva, jeho účinnosť a znížili (eliminovali) by aj eventuálnu toxicitu.

Kľúčové slová: perchlozón • *Mycobacterium tuberculosis* • rezistencia • MDR-TB • XDR-TB • koinfekcia HIV-1

Introduction

Tuberculosis (TB) is a communicable airborne infectious disease. Studies of human skeletons showed that this potentially deadly disease plagued humankind for millennia but its cause remained unknown until 1882, when Dr. Robert Koch announced discovery of a bacillus subsequently termed *Mycobacterium tuberculosis*[1, 2].

TB was regarded as one of relatively satisfactorily controlled infections for several decades, however, the emergence of drug-resistant tuberculosis (DR-TB) is becoming a major global threat[3]. Many forms of resistance of strains from *Mycobacterium* sp., including *M. tuberculosis*, to activity of drugs have been developed and can be found worldwide. The DR-TB form is caused by the *Mycobacterium* bacteria that are resistant to at least one first-line anti-TB drug, i.e., **isoniazid** (**INH**), **rifampicin** (**RIF**), **pyrazinamide** (**PZA**), or **ethambutol** (**EMB**). Polydrug-resistant TB (PDR-TB) indicates the resistance of the mycobacterial organisms to more than one anti-TB drug, but not including **INH** neither **RIF**. The MDR-TB disease is caused by the mycobacterial organisms resistant to at least **INH** and **RIF**. Original definition of extensively drug-resistant tuberculosis (XDR-TB) needs to be modified as all-oral regimens become the standard of care. Pre-extensively (pre-extremely) drug-resistant TB (pre-XDR-TB) is caused by the multidrug-resistant mycobacterial strains, which are, in addition, resistant to any **fluoroquinolone** (**FQ**) or second-line injectable agent, i.e., **amikacin** (**AK**), **kanamycin** (**KAN**), or **capreomycin** (**CAP**). The XDR-TB form is caused by the mycobacterial organisms, which show multidrug-resistance, and are resistant to any **FQ** and at least one of the second-line injectable anti-TB agents (**AK**, **KAN**, or **CAP**)[4].

Increasing incidence of MDR-TB and XDR-TB worldwide is a major concern for TB control programs[5–7]. In 2018, there were about half a million new cases of **RIF**-resistant TB (RR-TB), of which 78% had MDR-TB. Countries with the largest share of global burden were India (27%), China (14%) and the Russian Federation (9%)[3]. Globally, 3.4% of new TB cases and 18% of previously treated cases had MDR-TB, or RR-TB, with the highest proportions (> 50% in previously treated cases) in the countries of the former Soviet Union[3].

Regarding the fact that DR-TB notification rates relative to the population continue to increase in the Russian Federation despite the impressive decline in new TB notification rates[8], next sections of the paper provide a very brief overview on global situation together with possible promising non-pharmacotherapetic and especially pharmacotherapeutic interventions, including characterization and practical experiences with one original anti-TB drug developed in this country.

Current prevalence of tuberculosis in the Russian Federation

The prevalence of TB in some regions of the Russian Federation was characterized by a "certain" stabilization in the first decade of the 21st century. However, current overall situation remains very complicated and tense due to quite low level of prevention in adults together with worsening of clinical forms of both MDR-TB and XDR-TB[9].

Today's "Fight against Tuberculosis" programmes are heavily subsidized by the state and regional governments as well as health authorities. The Russian Federation is one of the largest and most ethnically diverse multi-national federations in the world. The fact is that each region, i.e., particular federal subjects (republics, oblasts (provinces), krais (territories), autonomous okrugs (areas), autonomous oblasts, or federal cities) and Federal Okrugs (subdivided into city okrugs and municipal raions), of the Russian Federation has its own specific characteristics and needs specific interventions[10–12].

Control of TB in some regions has been noticeably improved than in the others due to systematic and consistent implementation of fundamental principles of the TB control. The principles included rapid and reliable MDR-TB diagnostics, special chemotherapeutic strategy (including systematic registration of all TB cases, rehabilitation, multidrug chemotherapy schemes and provisions of support to overcome negative social stigma associated with the disease) as well as application of effective infection control practices[9–12].

More favorable epidemiological profile of TB burden in Tomsk Oblast might be attributed to highly effective anti-DR-TB programme, which was implemented in this region only. The programme has been recognized as one of the most successful projects to manage MDR-TB in the world. In contast, overall burden of TB in the Asian part of the Russian Federation is still very serious. The regions, where interventions are needed most urgently, are Chukotka Autonomous Okrug, Jewish Autonomous Oblast and Tyva Republic as well[9–12].

Continuous search for efficient and safe anti--tuberculosis drugs and vaccines

The aims of TB-treatment are to kill mycobacteria quickly, prevent the development and resistance to drugs,

eliminate lasting mycobacteria and prevent relapse and transmission of the disease[13], respectively. On the contrary, the tendency of mycobacteria to mutate and develop resistance to "simple" treatment is very common and was the reason for combined treatment of MDR-TB and XDR-TB, respectively[14]. Classification of the compounds, which were recommended by the World Health Organization (WHO) for use in both MDR-TB and XDR-TB regimens, can be found in reviews[6, 7].

In recent years, only a few effective drugs were approved and released for clinical practice in order to treat patients suffering from MDR-TB, XDR-TB, treatment-intolerant or non-responsive MDR-TB as a part of combination regimen[14–17].

Bedaquiline (**BDQ**; **1**), a molecule containing a diarylquinoline scaffold, **delamanid** (**DLM**; **2**), a nitro-dihydro-imidazooxazole derivative, and **pretomanid** (**PTM**; **3**), in whose structure a nitroimidazooxazine moiety is incorporated, represent new anti-TB agents. These compounds were approved by relevant authorities for using in clinical practice in different countries nearly 60 years after the last approval and release of a "classical" **RIF**. Two modern drugs, **BDQ** (**1**) and **DLM** (**2**), have been also registered in the Russian Federation[14–19].

The diarylquinoline derivative, **BDQ** (**1**) (Fig. 1), inhibits ATP generation in *M. tuberculosis* by interfering with its F-ATP synthase activity. Two mechanisms of action are broadly established for this molecule. Firstly, direct mechanism involves compound's binding to enzyme's *c*-ring to block its rotation, thus inhibiting ATP synthesis in enzyme's catalytic α3β3-headpiece. The process leads to depletion of bacterial ATP[20]. Secondly, indirect mechanism involves **BDQ**'s (**1**) uncoupling electron transport in the electron transport chain from ATP synthesis at the F-ATP synthase[21]. However, given uncoupler mechanism was not confirmed by recent findings of Sarathy et al. (2019)[22].

Another anti-TB agent approved recently, **DLM** (**2**) (Fig. 2), inhibits synthesis of mycobacterial cell wall components, methoxymycolic acid and ketomycolic acid. Mycolic acids are a complex mixture of branched, long-chain (C_{60}–C_{90}) fatty acids, representing key components of the highly hydrophobic cell wall[23]. The compound (**2**) is a prodrug, which gets activated by a specific enzyme, deazaflavin dependent nitroreductase (Rv3547). The reactive intermediate metabolite, formed between **DLM** (**2**) and the desnitro-imidazooxazole derivative, is considered to play a vital role in the inhibition of mycolic acid production[24].

Stover et al. (2000) suggested[25] that **PTM** (**3**) (Fig. 3) could act on the mycolic acid biosynthetic pathway *via* depletion of ketoymycolates and accumulation of hydroxymycolates, however, precise mechanism of its action is currently unclear. Baptista et al. (2018) proposed[26] that mentioned compound might generate a toxic metabolite, methylglyoxal, which damaged the pathogen.

The **PTM** (**3**) molecule in a combination with **BDQ** (**1**) and **linezolid** (**LNZ**; **4**) (Fig. 4) is approved for treating a limited and specific population of adult patients with XDR-TB, treatment-intolerant or non-responsive pulmonary MDR-TB[27].

Due to the mechanisms of action that are different from those of other available anti-TB drugs, efficacy of the compounds (**1**–**3**) appeared optimal in cases of the adults

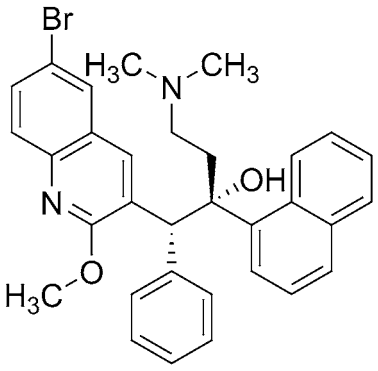

Fig. 1. Chemical structure of bedaquiline (BDQ; 1)

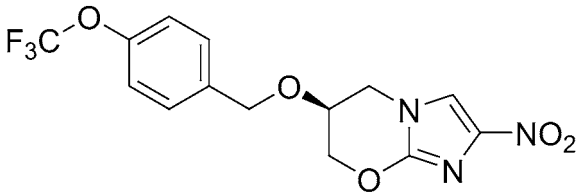

Fig. 3. Chemical structure of pretomanid (PTM; 3)

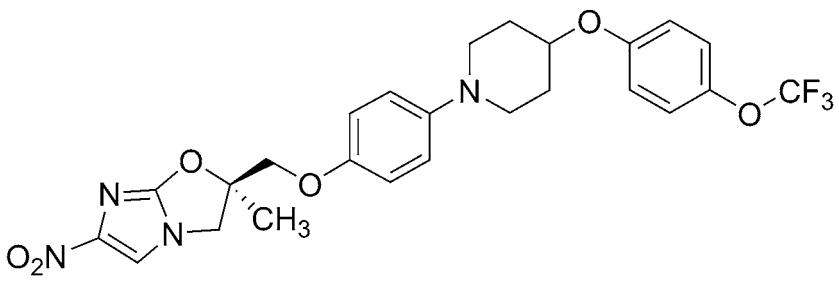

Fig. 2. Chemical structure of delamanid (DLM; 2)

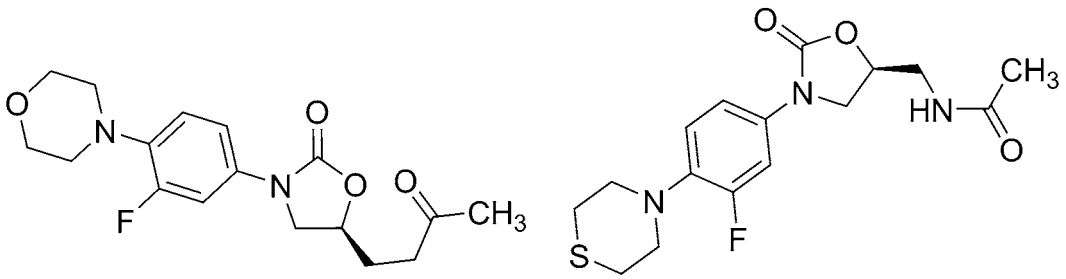

Fig. 4. Chemical structures of linezolid (LNZ; 4) and sutezolid (STZ; 5)

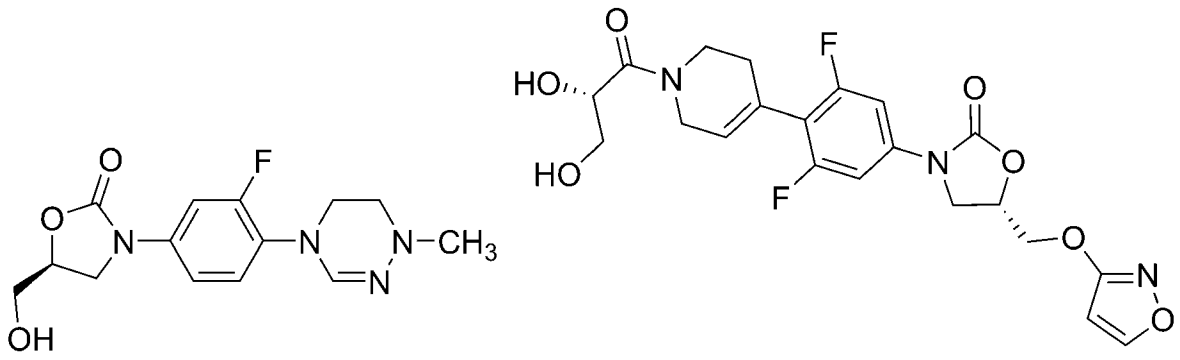

Fig. 5. Chemical structures of delpazolid (DPZ; 6) and posizolid (PSZ; 7)

with resistant pulmonary TB. Although drugs' pharmacokinetic and pharmacodynamic profiles seem optimal as well, potential cardiovascular side effects of **BDQ** (**1**) and **DLM** (**2**), such as QT-interval prolongation, have been associated with their use. Moreover, there can be found also some "technical" limitations of those modern anti-TB drugs, including high price and limited availability together with logistic issues in many regions of the world[14, 17].

Following those obstacles, the development, preclinical and clinical investigations, approval and clinical use of highly effective and safe anti-TB agents to combat DR-TB, MDR-TB, or XDR-TB, is still major challenge and task. **LNZ** (**4**), **sutezolid** (**STZ**; **5**), **delpazolid** (**DPZ**; **6**), **posizolid** (**PSZ**; **7**), or **contezolid** (**CTZ**; **8**) are currently in various phases of clinical trials. These compounds (Figs. 4, 5 and 6), which inhibit protein synthesis due to 50S ribosomal subunit blockage of mycobacterial pathogens, contain a differently substituted oxazolidinone structural motif[28].

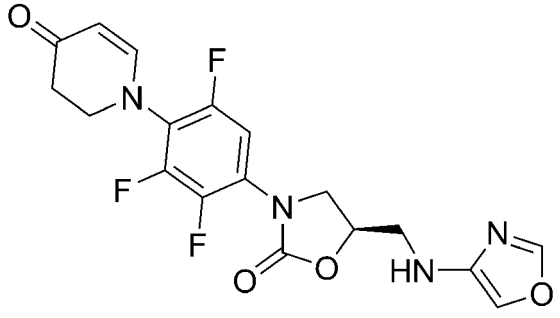

Fig. 6. Chemical structure of contezolid (CTZ; 8)

In addition, the patient-centered approach with suitable case management strategies should be implemented in practice, taking into very strong consideration social, cultural and environmental aspects of the care. All-oral treatment regimens including new and repurposed drugs might be a preferable strategy for most patients. Injectable anti-TB agents should be avoided whenever possible[6].

Development of a TB-vaccine also remains a critical global health priority given the deadly nature of ongoing TB-epidemic and spread of MDR-TB, or XDR-TB strains. Significant degree of protection against the TB disease shown by the **M72/AS01E** vaccine candidate, which is in a phase 2b clinical trial, strongly suggested that TB-vaccines are feasible and encouraging preclinical results from advanced vaccine candidates, such as a cytomegalovirus-vectored TB vaccine construct, offer the prospect of further progress[29].

Perchlozone as a new armor in combating multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis

Perchlozone (**PCZ**; **9**), chemically 4-tioureidoiminomethylpyridinium perchlorate, was synthesized in 1990 in the A. E. Favorsky Irkutsk Institute of Chemistry (Siberian Branch of the Russian Academy of Sciences), founded in 1957 as one of the first academic institutes

in the Eastern Siberia, in cooperation with St. Petersburg Research Institute of Phthisiopulmonology (Russian Federation). This compound (Fig. 7) belongs chemically into the class of **thiosemicarbazones**, whose anti-TB activity is well-known[30–32].

X-Ray diffraction analysis of a crystal structure of **PCZ** (**9**) showed that both pyridine and thiosemicarbazone fragments were almost planar. The cation part of given molecule contained four hydrogen atoms attached to nitrogens (*N*1, *N*3 and *N*4) (Fig. 7), which were capable of hydrogen bonding. Relatively weak hydrogen bonds involving sulfur atoms of neighboring thiosemicarbazone chains were present and linked the cations into "dimers"[33]. The hydrogen bond was formed between N-H (*N*3 atom of first monomer) and S (second monomer) as well as N-H (*N*3 atom of second monomer) and S (first monomer).

The molecular anti-TB mechanism for **thiosemicarbazones** has not been understood clearly yet. **Thiacetazone** (**TAZ**; **10**) (Fig. 8), also termed **thioacetazone**, **tibione**, or **amithiozone**, is an old, inexpensive anti-TB compound, which belongs structurally into the same "chemical" class as **PCZ** (**9**). It was proposed that **TAZ** (**10**), as a prodrug, required activation by a mycobacterially encoded monoxygenase, EthA. The activated compound affected mycolic acid synthesis, probably by inhibiting cyclopropane mycolic acid synthases. In addition, thiosemicarbazone group chelated metal cations strongly and anti-TB efficiency of **TAZ** (**10**) was potentiated by copper[23, 34, 35].

Therefore, anti-TB properties of **PCZ** (**9**) might be dependent on its conversion to one or more active agents. The EthA enzyme of *M. tuberculosis* would oxidize a "parent" compound to a highly reactive sulfenic acid form, which might specifically covalently react with a cysteine residue of a hydroxyacyl-ACP-dehydratase (HadA) subunit of dehydratase (type II fatty acid synthase; FAS-II), thereby inhibiting formation of hydroxyacyl--ACP-dehydratase heterodimers (HadAB) of FAS-II[36].

Moreover, a carbodiimide metabolite generated from a sulfenic acid intermediate *via* sulfenic acid form should react with mycothiol, a prinicipal thiol in mycobacteria, which protects the *M. tuberculosis* cell against oxidative damage and electrophilic toxins. Both sulfenic acid intermediate and carbodiimide metabolite might sensitize *M. tuberculosis* to oxidative damage[37].

The **PCZ (9)** molecule showed bactericidal activity *in vitro* against *M. tuberculosis* Erdman, *M. tuberculosis* $H_{37}R_v$, *M. tuberculosis* „Academia", *M. bovis bovinu*s 8 as well as 16 drug-resistant clinical isolates, including MDR-TB strains. Experiments employing animal models revealed therapeutic effect *in vivo* of **PCZ** (**9**) comparable to **INH**, **AK** and **ofloxacin** (**OFX**), respectively. In addition, **PCZ** (**9**) was even more efficient than **EMB** or **ciprofloxacin** (**CPX**) and was regarded as a more favorable therapeutic alternative than **FQs**. In summary, preclinical investigations of **PCZ** (**9**) confirmed its high activity *in vivo* against various TB and non-TB strains of mycobacteria as well as convenient pharmacokinetic and toxicological properties[30].

Clinical phases of the research started at the State Research Center for Preventive Medicine (Moscow; current name is the National Research Center for Preventive Medicine) and the State Research Institute of Immunology, Russian Academy of Medical Sciences (Moscow) in 2009[38, 39].

Upon official approval of **PCZ** (**9**) in November 2012 and its introduction in routine clinical practice for the treatment of MDR-TB and XDR-TB, the effectiveness of short-term (6-months) therapies was significantly increased[40, 41].

The use of concerned compound as an integral part of combination therapy regimens notably reduced the time of bacilli elimination in pulmonary TB caused by the DR-TB strains. It was observed that **PCZ** (**9**) gave patients suffering from the severest and epidemiogically poor form of TB a chance to recover[42].

When **PCZ** (**9**) was included in a scheme for the treatment of HIV-1 negative patients, who were infected with *M. avium*, anti-inflammatory effect of the drug was observed[42]. Similarly, if **BDQ** (**1**) and **PCZ** (**9**) were introduced simultaneously as active components of very complex treatment regimens for patients suffering from MDR-TB, or XDR-TB, ceasing of mycobacterial excretion, closing of decay cavities, regression of inflammatory changes, elimination of the TB bacilli and achieving of positive X-ray dynamics, respectively, was observed[43, 44].

Hypothyroidism is a well-known side effect of the MDR-TB treatment. However, only a few studies described an eventual relationship between the progression of this syndrome and anti-TB therapy based on **PCZ** (**9**). Results from a retrospective study involving the patients treated with **PCZ** (**9**) indicated that there was no need to replace given anti-TB drug with the other one(s) due to eventual worsening of hypothyroidism[45].

The thiosemicarbazone scaffold-containing molecule (**9**), when being combined with antiretroviral therapy,

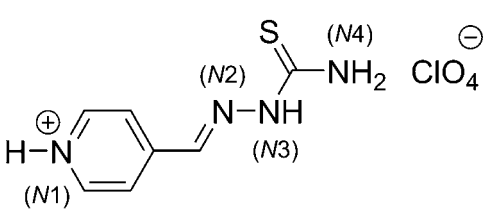

Fig. 7. Chemical structure of perchlozone (PCZ; 9)

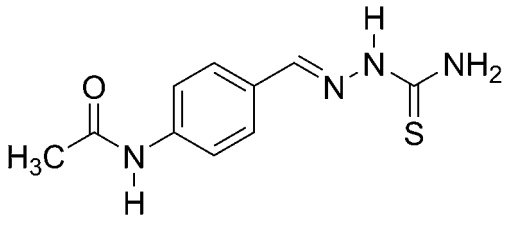

Fig. 8. Chemical structure of thiacetazone (TAZ; 10)

was able to increase the number of CD4+ cells and concentration of a specific interleukin (IL-4), thus providing good prognostic value for the treatment of patients with HIV-1 and lung TB co-infection[46]. In addition, the inclusion of both **BDQ** (**1**) and **PCZ** (**9**) in complex chemotherapy of MDR-TB and XDR-TB in HIV-1 positive patients increased significantly the effectiveness of such treatment in terms of stopping mycobacterial excretion and closing decay cavities[47].

Toxicity of **PCZ** (**9**) and its tolerability by the human organism were evaluated in relevant preclinical and clinical studies and all those parameters were acceptable, as published[39]. However, recent preliminary conclusions of a research team from the East-Siberian Institute of Medical and Ecological Research in Angarsk (Russian Federation), who aimed reactions *in vivo* of experimental (animal) models to subacute inhalation exposure of given drug, indicated compound's genotoxicity[48].

Besides, serious *in vitro* cytotoxicity issues were connected with other very promising anti-TB compounds, which contained a (substituted) thiosemicarbazone moiety[31]. Therefore, none of these substances was tested *in vivo*.

Nanoparticle-based drug formulations might be a promising way of how to avoid such toxicological obstacles. If experimental (animal) models suffering from MDR-TB were treated with intravenous immunoglobulin G (IgG)--modified nanoparticles and intraperitoneal IgG-modified microparticles, both loaded with **PCZ** (**9**), targeted particle delivery to the foci of infection in TB-animals was seen. Phagocytic macrophages of experimental animals internalized the particles and transported them to the foci of TB in inner organs. In addition, eventual toxicity of the drug was considerably reduced[49].

Gopal and Dick (2015) found out that **PCZ** (**9**) and **TAZ** (**10**) affected a similar spectrum of mycobacterial strains and, in addition, might show a similar mechanism of action. The authors noted that possible cross-resistance of **PCZ** (**9**) and **TAZ** (**10**) with **ethionamide** (**ETA**) *via* EThA mutations should be kept in mind[50].

The main limitation, which prevents more extensive use of **PCZ** (**9**) in the therapy of MDR-TB or XDR-TB, is that considered drug is practically not available outside the Russian Federation and has not been included in current recommendations of WHO, as published[6, 7, 14].

Conclusions

The progress, which was achieved in the anti-TB compounds development together with consistent implementation of effective anti-TB programmes in the 21st century, formed preconditions for satisfactory management of TB in the world. However, it might be quite irresponsible to speak silently about a very close victory over the disease or even declare loudly the winning approaches. Following notable increase in the number of patients suffering from MDR-TB, or XDR-TB worldwide who could be, in addition, co-infected with HIV-1, the use of **PCZ** (**9**), as a very efficient and safe component of combination therapy regimens, improved markedly therapeutic and clinical outcomes of the treatment. It might be expected that **PCZ** (**9**) acted as a prodrug. Baeyer-Villiger monooxygenase (EthA) from *Mycobacterium* spp. has broad substrate specificity, being able to oxidize different compounds, including the ones containing a thiocarbamide, or thiosemicarbazone moiety. Thus, both highly reactive sulfenic acid intermediate and carbodiimide metabolite of **PCZ** (**9**) inhibit mycobacterial cell wall synthesis interfering with a dehydration step of the type II fatty acid synthase (FAS II) pathway and sensitize the *M. tuberculosis* cell to oxidative stress, respectively. Recent preliminary findings could indicate possible genotoxicity *in vivo* when experimental (animal) models were exposed to subacute inhalation of the drug. Therefore, it might be very reasonable to continue in more thorough and precise research focused on the selection of suitable formulations of **PCZ** (**9**). In this regard, nano- or microparticle-based drug formulations might be taken into strong consideration in order to eliminate (eventual) toxicity of this very powerful synthetic "weapon" against MDR-TB and XDR-TB as well.

Acknowledgements

We would like to thank the Czech and Slovak Pharmacy Journal (Česká a slovenská farmacie) for the opportunity to publish the article.

Conflict of interest: none.

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