

REVIEW ARTICLE

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

Pohl'ad na perchlozón, antituberkulotikum z Ruskej federácie

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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 monooxygenase (EthA). The activated forms might act in multiple ways, including inhibition of mycobacterial cell wall synthesis due to interference 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 relevant 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. **Perchlozón** 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 senzitivizácie 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 genotoxickú 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 ciele 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 threat³. 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 pre-

viously treated cases had MDR-TB, or RR-TB, with the highest proportions (> 50% in previously treated cases) in the countries of the former Soviet Union³.

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 rates⁸, next sections of the paper provide a very brief overview on global situation together with possible promising non-pharmacotherapeutic 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-TB⁹.

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 contrast, 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 disease¹³, 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, respectively¹⁴. 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 $\alpha 3\beta 3$ -headpiece. The process leads to depletion of bacterial ATP²⁰. Secondly, indirect mechanism involves **BDQ's (1)** uncoupling electron transport in the electron transport chain from ATP synthesis at the F-ATP synthase²¹. 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 wall²³. 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 production²⁴.

Stover et al. (2000) suggested²⁵ 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) proposed²⁶ 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-TB²⁷.

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

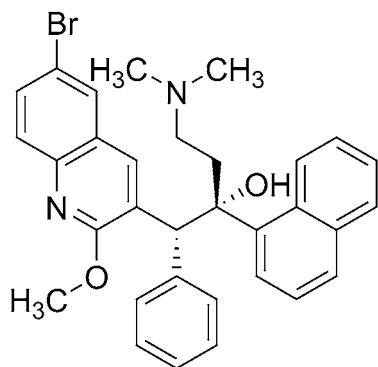


Fig. 1. Chemical structure of bedaquiline (BDQ; 1)

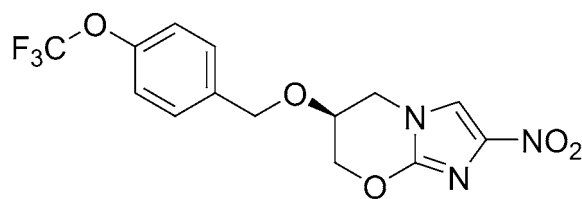


Fig. 3. Chemical structure of pretomanid (PTM; 3)

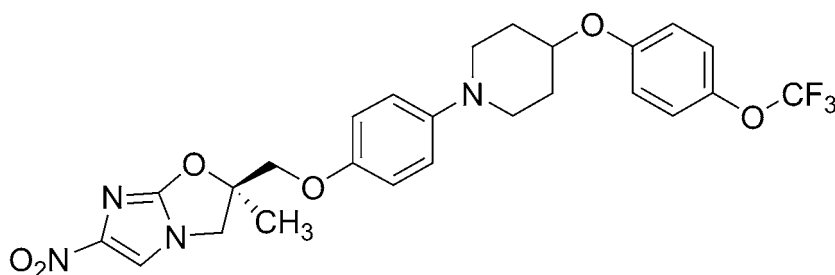


Fig. 2. Chemical structure of delamanid (DLM; 2)

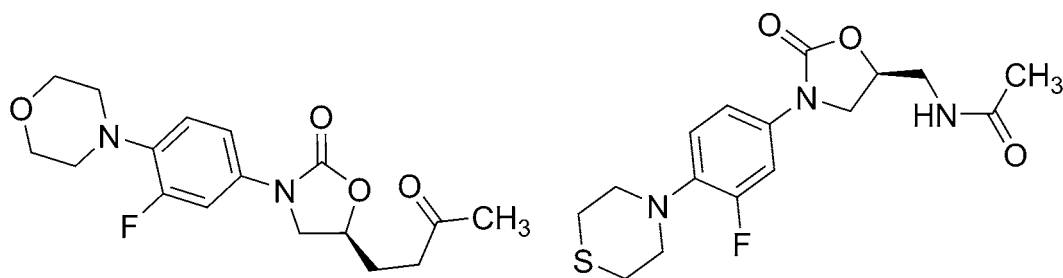


Fig. 4. Chemical structures of linezolid (LNZ; 4) and sutezolid (STZ; 5)

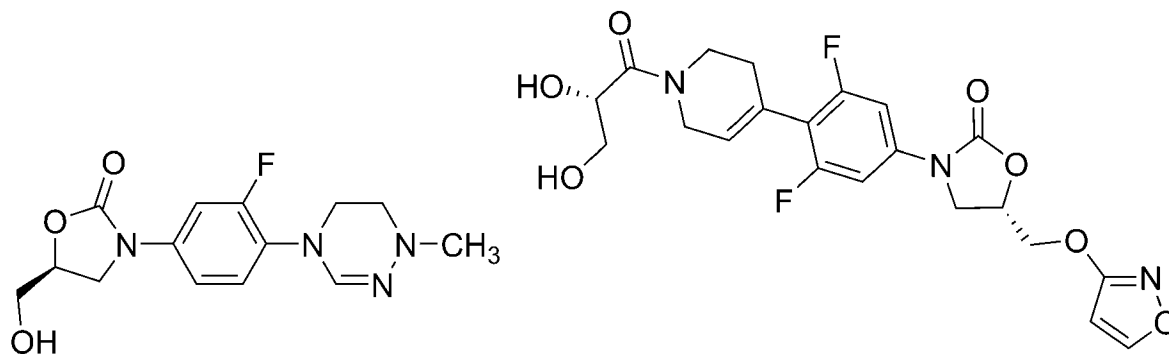


Fig. 5. Chemical structures of delpazolid (DPZ; 6) and posizolid (PSZ; 7)

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 motif²⁸.

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 possible⁶.

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 progress²⁹.

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

Perchlozone (PCZ; 9), chemically 4-tioureidoimino-methylpyridinium 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

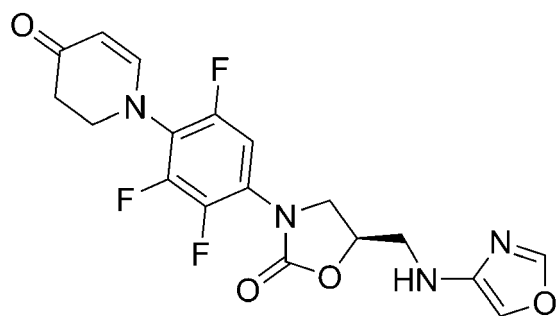


Fig. 6. Chemical structure of contezolid (CTZ; 8)

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 (*N1*, *N3* and *N4*) (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 (*N3* atom of first monomer) and S (second monomer) as well as N-H (*N3* 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 monooxygenase, 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-II³⁶.

Moreover, a carbodiimide metabolite generated from a sulfenic acid intermediate *via* sulfenic acid form should react with mycothiol, a principal 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 damage³⁷.

The **PCZ (9)** molecule showed bactericidal activity *in vitro* against *M. tuberculosis* Erdman, *M. tuberculosis* H₃₇R_v, *M. tuberculosis* „Academia“, *M. bovis bovinus*

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 properties³⁰.

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 epidemiologically poor form of TB a chance to recover⁴².

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 observed⁴². 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 hypothyroidism⁴⁵.

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

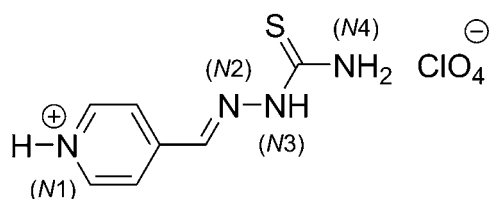


Fig. 7. Chemical structure of perchlozone (PCZ; 9)

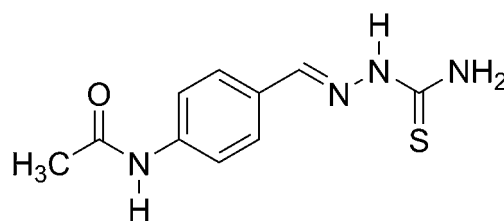


Fig. 8. Chemical structure of thiacetazone (TAZ; 10)

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-infection⁴⁶⁾. 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 cavities⁴⁷⁾.

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 published³⁹⁾. 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 genotoxicity⁴⁸⁾.

Besides, serious *in vitro* cytotoxicity issues were connected with other very promising anti-TB compounds, which contained a (substituted) thiosemicarbazone moiety³¹⁾. 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 reduced⁴⁹⁾.

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 mind⁵⁰⁾.

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 mono-oxygenase (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.

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Conflict of interest: none.

References

1. Vasava M. S., Bhoi M. N., Rathwa S. K., Borad M. A., Nair S. G., Patel H. D. Drug development against tuberculosis: Past, present and future. *Indian J. Tuberc.* 2017; 64, 252–275. doi: 10.1016/j.ijtb.2017.03.002
2. Hershkovitz I., Donoghue H. D., Minnikin D. E., May H., Lee O. Y., Feldman M., Galili E., Spigelman M., Rothschild B. M., Bar-Gal G. K. Tuberculosis origin: the Neolithic scenario. *Tuberculosis* 2015; 95(Suppl. 1), S122–S126. doi: 10.1016/j.tube.2015.02.021
3. World Health Organization (WHO). Global Tuberculosis Report 2019. Geneva: World Health Organization 2019; 297.
4. Chetty S., Ramesh M., Singh-Pillay A., Soliman M. E. S. Recent advancements in the development of anti-tuberculosis drugs. *Bioorg. Med. Chem. Lett.* 2017; 27, 370–386. doi: 10.1016/j.bmcl.2016.11.084
5. Koch A., Cox H., Mizrahi V. Drug-resistant tuberculosis: Challenges and opportunities for diagnosis and treatment. *Curr. Opin. Pharmacol.* 2018; 42, 7–15. doi: 10.1016/j.coph.2018.05.013
6. Mase S. R., Chorba T. Treatment of drug-resistant tuberculosis. *Clin. Chest Med.* 2019; 40, 775–795. doi: 10.1016/j.ccm.2019.08.002
7. Tiberi S., Zumla A., Battista Migliori G. Multidrug and extensively drug-resistant tuberculosis. Epidemiology, clinical features, management and treatment. *Infect. Dis. Clin. North. Am.* 2019; 33, 1063–1085. doi: 10.1016/j.idc.2019.09.002
8. Dadu M., Hovhannesyan A., Ahmedov S., van der Werf M. J., Dara M. Drug-resistant tuberculosis in Eastern Europe and Central Asia: a time-series analysis of routine surveillance data. *Lancet Infect. Dis.* 2020; 20, 250–258. doi: 10.1016/S1473-3099(19)30568-7

9. Belyaeva E., Chernokhaeva I., Sapozhnikova N., Nazarenko M., Starshinova A., Yablonskiy P. Factors predisposing to the development of extensive drug resistance of *Mycobacterium tuberculosis*. Medicinal Alliance (Medicinskiy Alyans; In Russian) 2017; 5, 51–56.
10. Ergeshov A. E. Tuberculosis in the Russian Federation: Situation, challenges and perspectives. Annals of the Russian Academy of Medical Sciences (Vestnik RAMN; In Russian) 2018; 73, 330–337. doi: 10.15690/vramn1023
11. Meshkov I., Petrenko T., Keiser O., Estill J., Revyakina O., Felker I., Raviglione M. C., Krasnov V., Schartz Y. Variations in tuberculosis prevalence, Russian Federation: a multivariate approach. Bull. World Health Organ 2019; 97, 737–745. doi: 10.2471/BLT.19.229997
12. Yablonskii P. K., Vizel A. A., Galkin V. B., Shulgina M. V. Tuberculosis in Russia. Its history and its status today. Am. J. Respir. Crit. Care Med. 2015; 191, 372–376. doi: 10.1164/rccm.201305-0926OE
13. Augustynowicz-Kopeć E., Demkow U., Grzelewska-Rzymowska I., Korzeniewska-Kosela M., Langfort R., Michalowska-Mitczuk D., Rowińska-Zakrzewska E., Zielonka T. M., Ziolkowski J., Zwolska Z. Guidelines of Polish Respiratory Society concerning diagnosis, treatment and prevention of tuberculosis in adults and in children. Pneumol. Alergol. Pol. 2013; 81, 323–379.
14. Zellweger J.-P. Current options for the management of multi-drug-resistant tuberculosis (review). Medicinal Alliance (Medicinskiy Alyans; In Russian) 2017; 6, 22–28.
15. Ryan N. J., Lo J. H. Delamanid: First global approval. Drugs 2014; 74, 1041–1045. doi: 10.1007/s40265-014-0241-5
16. Keam S. J. Pretomanid: First approval. Drugs 2019; 79, 1797–1803. doi: 10.1007/s40265-019-01207-9
17. Esposito S., Bianchini S., Blasi F. Bedaquiline and delamanid in tuberculosis. Expert Opin. Pharmacother. 2015; 16, 2319–2330. doi: 10.1517/14656566.2015.1080240
18. Sensi P. History of the development of rifampin. Rev. Infect. Dis. 1983; 5(Suppl. 3), S402–S406. doi: 10.1093/clinids/5.supplement_3.s402
19. Delytba® (delamanid) approved in Russian Federation for treatment of pulmonary multidrug-resistant tuberculosis (MDR-TB). https://www.otsuka.co.jp/en/company/newsreleases/2020/20200525_1.html (20.9.2020)
20. Haagsma A. C., Podasca I., Koul A., Andries K., Guillemont J., Lill H., Bald D. Probing the interaction of the diarylquinoline TMC207 with its target mycobacterial ATP synthase. PLoS One 2011; 6, art. no. e23575 (7 pp.). doi: 10.1371/journal.pone.0023575
21. Hards K., Robson J. R., Berney M., Shaw L., Bald D., Koul A., Andries K., Cook G. M. Bactericidal mode of action of bedaquiline. J. Antimicrob. Chemother. 2015; 70, 2028–2037. doi: 10.1093/jac/dkv05
22. Sarathy J. P., Gruber G., Dick T. Re-understanding the mechanisms of action of the anti-mycobacterial drug bedaquiline. Antibiotics (Basel) 2019; 8, art. no. 261 (12 pp.). doi: 10.3390/antibiotics8040261
23. Alahari A., Trivelli X., Guérardel Y., Dover L. G., Besra G. S., Sacchettini J. C., Reynolds R. C., Coxon G. D., Kremer L. Thiacetazone, an antitubercular drug that inhibits cyclopropanation of cell wall mycolic acids in mycobacteria. PLoS One 2007; 2, art. no. e1343 (12 pp.). doi: 10.1371/journal.pone.0001343.
24. Xavier A. S., Lakshmanan M. Delamanid: A new armor in combating drug-resistant tuberculosis. J. Pharmacol. Pharmacother. 2014; 5, 222–224. doi: 10.4103/0976-500X.136121
25. Stover C. K., Warren P., van Deanter D. R., Sherman D. R., Arain T. M., Langhorne M. H., Anderson S. W., Towell J. A., Yuan Y., McMurray D. N., Kreiswirth B. N., Barry C. E., Baker W. R. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 2000; 405, 962–966. doi: 10.1038/35016103
26. Baptista R., Fazakerley D. M., Beckmann M., Baillie L., Mur L. A. J. Untargeted metabolomics reveals a new mode of action of pretomanid (PA-824). Sci. Rep. 2018; 8, art. no. 5084 (7 pp.). doi: 10.1038/s41598-018-23110-1
27. Conradie F., Diacon A. H., Ngubane N., Howell P., Everitt D., Crook A. M., Mendel C. M., Egizi E., Moreira J., Timm J., McHugh T. D., Wills G., Bateson A., Hunt R., van Niekerk Ch., Li M., Olugbosi M., Spigelman M., on behalf of Nix-TB Trial Team. Bedaquiline, pretomanid and linezolid for treatment of extensively drug resistant, intolerant or non-responsive multidrug resistant pulmonary tuberculosis. N. Engl. J. Med. 2020; 382, 893–902. doi: 10.1056/NEJMoa1901814
28. Reddy Tetali S., Kunapaeddi E., Prasad Mailavaram R., Singh V., Borah P., Deb P. K., Venugopala K. N., Hourani W., Tekade R. K. Current advances in the clinical development of anti-tubercular agents. Tuberculosis 2020; 125, art. no. 101989 (12 pp.). doi: 10.1016/j.tube.2020.101989
29. Schrager L. K., Vekemens J., Drager N., Lewinsohn D. M., Olesen O. F. The status of tuberculosis vaccine development. Lancet Infect. Dis. 2020; 20, 28–37. doi: 10.1016/S1473-3099(19)30625-5
30. Pavlova M., Vinogradova T., Starshinova A., Sapozhnikova N., Chernokhaeva I., Archakova L., Yablonskii P. Confirmation of preclinical studies data on thioureidoiminomethylpyridinium perchlorate (perchlozon) in clinic. Int. J. Curr. Res. 2015; 7, 14501–14505.
31. Opletalová V., Doležel J. Thiosemicarbazones and their antimycobacterial effects. Čes. slov. Farm. 2013; 62, 78–83.
32. Pavan F. R., da Silva Maia P. I., Leite S. R. A., Defflon V. M., Batista A. A., Sato D. N., Franzblau S. G., Leite C. Q. F. Thiosemicarbazones, semicarbazones, dithiocarbazates and hydrazide/hydrazones: anti-*Mycobacterium tuberculosis* activity and cytotoxicity. Eur. J. Med. Chem. 2010; 45, 1898–1905. doi: 10.1016/j.ejmech.2010.01.028
33. Smolentsev A. I., Lavrenova L. G., Elokhina V. N., Nakhmanovich A. S., Larina L. L. Crystal structure of pyridine-4-aldehyde thiosemicarbazone perchlorate and trifluoromethane sulfonate. J. Struct. Chem. 2009; 50, 500–504. doi: 10.1007/s10947-009-0076-1
34. Volynets G. P., Tukalo M. A., Bdzholia V. G., Derkach N. M., Gumeniuk M. I., Tarnavskiy S. S., Starosyla S. A., Yarmoluk S. M. Benzaldehyde thiosemicarbazone derivatives against replicating and nonreplicating *Mycobacterium tuberculosis*. J. Antibiot. (Tokyo) 2019; 72, 218–224. doi: 10.1038/s41429-019-0140-9
35. Dover L. G., Alahari A., Grattraud P., Gomes J. M., Bhowruth V. E. EthA, a common activator of thiocarbamide-containing drugs acting on different mycobacterial targets. Antimicrob. Agents Chemother. 2007; 51, 1055–1063. doi: 10.1128/AAC.01063-06
36. Grzegorzewicz A. E., Eynard N., Quémard A., North E. J., Margolis A., Lindenberger J. J., Jones V., Korduláková J.,

- Brennan P. J., Lee R. E., Ronning D. R., McNeil M. R., Jackson M. Covalent modification of the *Mycobacterium tuberculosis* FAS-II dehydratase by Isoxyl and Thiacetazone. *ACS Infect. Dis.* 2015; 1, 91–97. doi: 10.1021/id500032q
37. Qian L., Ortiz de Montellano P. R. Oxidative activation of thiacetazone by the *Mycobacterium tuberculosis* flavin monooxygenase EtaA and human FMO1 and FMO3. *Chem. Res. Toxicol.* 2006; 19, 443–449. doi: 10.1021/tx050328b
 38. Vinogradova T. I., Zabolotnykh N. V., Vasilyeva S. N., Vitovskaya M. L., Malygina E. I. Development of optimal schemes of drug-resistant tuberculosis therapy in experiment. *Biology and Experimental Medicine (Biologia i Experimentalnaya Medicina; In Russian)* 2011; 28, 88–93.
 39. Yablonskiy P. K., Vinogradova T. I., Levashev Y. N., Pavlova M. V., Zilber E. K., Starshinova A. A., Sapozhnikova N. V., Chernokhaeva I. V., Archakova L. I., Zabolotnykh N. V., Vitovskaya M. L. Preclinical and clinical trials of the new tuberculosis drug perchlozone. *Therapeutic Archive (Terapevticheskii Arkhiv; In Russian)* 2016; 88, 111–115. doi: 10.17116/terarkh2016883111-115
 40. Chernokhaeva I. V., Pavlova M. V., Starshinova A. A., Belyaeva E. N., Sapozhnikova N. V., Gavrilov P. V., Zhuravlev V. Yu., Archakova L. I., Yablonskiy P. K. Efficacy of treatment by perchlozone of multidrug-resistant tuberculosis. *Practical Medicine (Prakticheskaya Medicina; In Russian)* 2015; 1, 81–85.
 41. Istomina E., Sokolovich E., Pavlova M., Archakova L., Sapozhnikova N., Chernokhaeva I., Yablonskiy P. Clinical example of effective treatment of the recurrence of tuberculosis with multiple dosing stability of mycobacteria. *Medicinal Alliance (Medicinskiy Alyans; In Russian)* 2019; 7, 57–64.
 42. Starshinova A., Belyaeva E., Chernokhaeva I., Sapozhnikova N., Pavlova M., Nazarenko M., Esmedlyeva D., Diyakova M., Yablonskiy P. Inflammatory response in patients with multiply drug resistant lung tuberculosis against the background of treatment including thioreidoiminomethylpyridinium perchlorate. *Medicinal Alliance (Medicinskiy Alyans; In Russian)* 2019; 7, 24–31.
 43. Pavlova M. V., Ershova E. S., Sapozhnikova N. V., Starshinova A. A., Archakova L. I., Chernokhaeva I. V. A clinical case of effective treatment of extensive drug resistance tuberculosis with new anti-tuberculosis drugs. *Tuberculosis and Lung Diseases (Klinicheskoe Nablyudeni; In Russian)* 2017; 95, 58–62. doi: 10.21292/2075-1230-2017-95-12-58-62
 44. Pavlova M. V., Vinogradova T. I., Zabolotnykh N. V., Ershova E. S., Sapozhnikova N. V., Chernokhaeva I. V., Archakova L. I., Vitovskaya M. L., Dogonadze M. Z., Starshinova A. A., Grishko A. N., Yablonskiy P. K. Prospects for the use of new generation of anti-tuberculosis drugs in treatment of drug-resistant tuberculosis (experimental and clinical study). *Rev. Clin. Pharmacol. Drug Ther.* 2018; 16, 33–40. doi: 10.17816/RCF16433-40
 45. Potanina O. Hypothyroidism in tuberculosis patients receiving thioureidoiminomethylpyridinium perchlorate therapy. *Medicinal Alliance (Medicinskiy Alyans; In Russian)* 2018; 6, 41–45.
 46. Strygin A. V., Klyausov A. S., Dotsenko A. M., Strygina A. O., Morkovin E. I. Effects of perchlozone and antibodies against human gamma-interferon and CD4 glycoprotein on the immune system of HIV-infected patients with associated pulmonary tuberculosis. *Journal of VolgSMU (Vestnik VolgSMU; In Russian)* 2017; 62, 42–46. doi: 10.19163/1994-9480-2017-2(62)-42-46
 47. Pavlova M., Ershova E., Sapozhnikova N., Chernokhaeva I., Starshinova A., Anisimova A., Yablonskiy P. The first experience of bedaquiline and thioureidoiminomethylpyridinium perchlorate combination in patients with HIV infection. *Medicinal Alliance (Medicinskiy Alyans; In Russian)* 2019; 7, 22–29.
 48. Sosedova L. M., Vokina V. A., Kapustina E. A., Bogomolova E. S. Evaluation of genotoxicity of perchlozone, antituberculous drug. *Bull. Exp. Biol. Med.* 2020; 169, 48–52. doi: 10.1007/s10517-020-04821-w
 49. Churilov L., Korzhikov-Vlakh V., Sinitsyna E., Polyakov D., Darashkevich O., Poida M., Platonova G., Vinogradova T., Utekhin V., Zabolotnykh N., Zinserling V., Yablonskiy P., Urtti A., Tennikova T. Enhanced delivery of 4-thioureidoiminomethylpyridinium perchlorate in tuberculosis models with IgG functionalized poly(lactic acid)-based particles. *Pharmaceutics* 2019; 11, art. no. 2 (20 pp.). doi: 10.3390/pharmaceutics11010002
 50. Gopal P., Dick T. The new tuberculosis drug Perchlozone shows cross-resistance with thiacetazone. *Int. J. Antimicrob. Agents.* 2015; 45, 430–433. doi: 10.1016/j.ijantimicag.2014.12.026