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SEARCH FOR NEW ANTITUBERCULAR DRUGS AMONG THE SALTS AND BASES OF O-AROYLATION PRODUCTS OF β-(THIOMORFOLIN-1-YL)- AND β-(4-METHYLPIPERAZIN-1-YL)PROPIOAMIDOXIMES

Abstract. Tuberculosis (TB) is the leading cause of death and morbidity in more than one third of the world's population. The incidence of tuberculosis in Kazakhstan over the past 10 years has decreased to 52,2 per 100 thousand population (2017) against 126,4 per 100 thousand population in 2007; mortality rate – in 6 times, reaching 3,0 per 100 thousand of population (2017). In order to reduce the duration of treatment, exclude the rapid development of drug resistance, toxic and side effects of existing anti-TB drugs and to reduce the cost of extremely expensive treatment of TB (DS, MDR, XDR), the world is searching for new TB drugs. We have synthesized the salts and bases of the O-aroylation products of β-(thiomorpholin-1-yl) and β-(4-methylpiperazin-1-yl) propioamidoximes, containing in the β-position pharmacophore fragments of 1-methylpiperazine and thiomorpholine. *In vitro* anti-tuberculosis screening of β-aminopropioamidoxime derivatives in the DS, DR and MDR strains of *M. tuberculosis* revealed highly active competitive compounds which are less toxic than rifampicin and isoniazid with activity significantly exceeding the activity of the reference preparations. It is assumed that these compounds may be the subject of subsequent trials in the development of doses and new treatment regimens for TB.

Key words: tuberculosis; hydrochlorides and bases of O-aroyl-β-(thiomorpholin-1-yl) propioamidoximes; hydrochloride, iodomethylate of β-(4-methylpiperazin-1-yl) propioamidoxime; screening for DS, DR, and MDR strains of *M. tuberculosis*; Shkolnikova liquid medium; the average subcutaneous toxicity of O-aroyl-β-aminopropioamidoximes.

Introduction. Tuberculosis (TB) is the leading cause of death and morbidity in more than one third of the world's population. Of the 56,4 million deaths worldwide due to the 10 leading causes in 2016, tuberculosis ranked 10th, from which 1,4 million people died [1].

The incidence of tuberculosis in Kazakhstan over the past 10 years has decreased by 2,4 times, reaching 52,2 per 100 thousand in 2017 against 126,4 per 100 thousand of the population in 2007 and the death rate by 6 times, reaching 3,0 per 100 thousand population [2].

In May 2014, the World Health Organization (WHO) approved a new global TB control strategy «End TB». This strategy marks a critical shift from tuberculosis control to ending the epidemic by 2035. The «End TB» strategy emphasizes the need for innovation to accelerate progress by optimizing existing ones in the short term and introducing of new innovative modes in the long term [3].

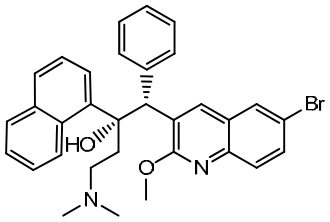
According to WHO, in 2017, 10 million people became sick with tuberculosis, and 1,6 million people (including 0,3 million people with HIV) died from the disease.

Tuberculosis control in Kazakhstan is a priority and is carried out within the framework of the State Health Development Program «Densaulyk» for 2016–2020. In 2018, 42 billion tenge was allocated from the state budget for social assistance, diagnosis and treatment for patients with tuberculosis [4].

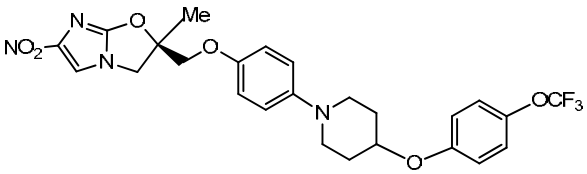
Currently, there are several clinical studies on the evaluation of repurposed and new anti-TB drugs, funded mainly by USAID, the Union Against Tuberculosis and Lung Diseases, the UK Medical Research Council, the non-governmental organization «Partners in Health», etc. [5].

One of the «End TB (Expand New Drugs for TB)» projects aims to increase access to effective and user-friendly treatment regimens for patients with MDR (multidrug-resistant) TB and XDR (wide-drug-resistant) TB in 17 countries: Armenia, Bangladesh, Belarus, Ethiopia, Georgia, Indonesia, Kazakhstan, Kenya, Kyrgyzstan, Lesotho, Myanmar, Nepal, North Korea, Pakistan, Haiti, South Africa and Peru. The study involved at least 2,600 patients. In Kazakhstan, the implementation of «End TB project» began after the signing of a Memorandum of Cooperation between the non-governmental organization «Partners in Health» and the Ministry of Health and Social Development of the Republic of Kazakhstan on October 27, 2015, which resulted in approval for treating a cohort of 600 patients with MDR TB and XDR TB during 2016–2019 [6].

There are more than 370 people on the treatment. Regimens including new drugs (Bedaquiline and Delamanid) are introduce in the nine regions of Kazakhstan. By the results of five months, the percentage of those cured makes up almost all 100. Bedaquiline and delamanid have been developed in the recent years. WHO issued interim recommendations on the use of these drugs in 2013 and 2014 [7, 8].



Bedaquiline (TMC207)

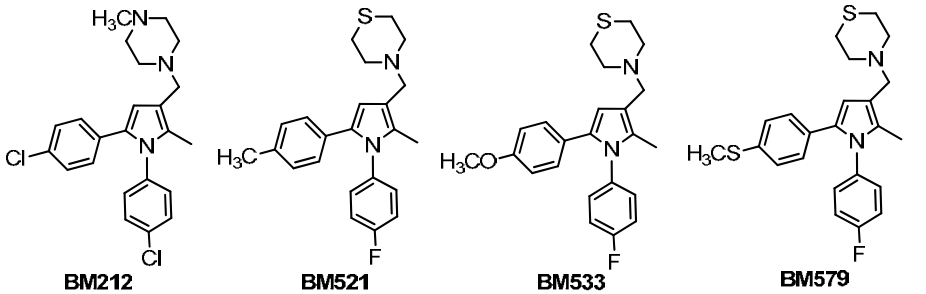


Delamanid (ORC-67683)

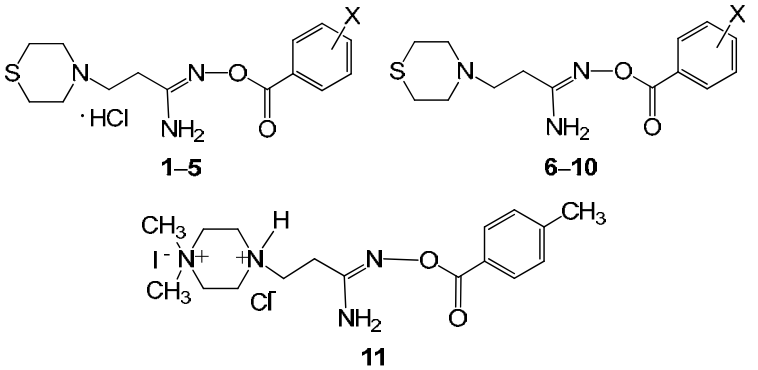
A search for qualitatively new anti-tuberculosis drugs with the requirements of reducing the duration of treatment, eliminating of the rapid drug resistance development and toxic side effects of the existing anti-tuberculosis drugs and reducing the cost of extremely expensive treatment of TB (DS, MDR, XDR) is conducting in the world.

1,5-Diphenylpyrroles have been identified as a class of compounds with high *in vitro* anti-tuberculosis activity. Replacing of the methylpiperazine substituent

for thiomorpholine and replacing the chlorine atom in position 4 of the N-phenyl moiety with the fluorine atom, as well as varying the aromatic substituents at the C-2 atom of the pyrrole ring during the transition from *para*-CH_3 (BM221) to *para*-CH_3O (BM233), and to *para*-CH_3S (BM579) in 1,5-(4-chlorophenyl)-2-methyl-3-(4-methylpiperazin-1-yl)methyl-1H-pyrrole (BM212) leads to an increase in *in vitro* anti-tuberculosis activity on *M. tuberculosis* H37Rv strains [9–11]:



Results and discussion. Taking into account the above examples [9–11], we synthesized compounds of the β-aminopropioamidoxime series containing in the β-position fragments of 1-methylpiperazine and thiomorpholine (**1**–**11**) [12, 13]:



X = *p*-CH_3O (**1**, **6**), *p*-CH_3 (**2**, **7**), H (**3**, **8**), *p*-Br (4, **9**), *m*-Cl (**5**, **10**)

In vitro anti-tuberculosis screening of a series of O-aroyl-β-aminopropioamidoximes (**1**–**11**) on DS museum H37Rv and wild* I MTB strains and two wild DR and MDR strains of MBT II and III on Shkolnikova liquid medium found that compounds **1**–**11** in varying degree have anti-tubercular activity from > 100 to 0,01 μg/ml (table 1).

* Wild strains of M. tuberculosis I, II, III were isolated from the patients and typed in the RSE «National Scientific Center for Phthisiopulmonology of the Republic of Kazakhstan» of the Ministry of Health of the Republic of Kazakhstan: I – DS (drug-sensitive) to anti-TB drugs, II – DR (drug-resistant) to rifampicin, III – MDR to rifampicin, isoniazid and ethambutol.

Table 1 – Bactericidal activity (MBC – minimal bactericidal concentration) and average subcutaneous toxicity (LD_{50}) of O-aroyl-β-(thiomorpholin-1-yl)propioamidoximes (**1**–**10**) and double salt of O-*para*-toluoyl-(4-methylpiperazin-1-yl) propioamidoxime (**11**) on DS and DR strains of *M. tuberculosis*

# comp.	MBC on the *M. tuberculosis* strains, μg/ml				
	H37Rv	I	II	III	LD_{50}, mg/kg
1	>100	>100	100	100	–
2	100	100	100	100	–
3	100	100	100	100	–
4	>100	>100	100	100	–
5	100	100	100	100	–
6	100	100	100	100	–
7	>100	>100	>100	>100	–
8	0,01	0,01	100	100	325,0±17,8
9	>20	>20	100	100	–
10	100	100	100	100	–
11	0,01	0,01	0,1	0,1	1750,0 ± 35,6
Rifampicin	1	1	2	2	267,6±7,2
Isoniazid	0,1	0,1	1	1	62,5±12,8

Thus, on the DS strains of MTB O-benzoyl-β-(thiomorpholin-1-yl)propioamidoxime (**8**) and hydrochloride, iodomethylate of O-*para*-toluoyl-β-(1-methylpiperazin-1-yl)propioamidoxime (**11**) showed the highest activity at 0,01 μg/ml; compound **9** had an average anti-tuberculosis activity with MBC > > 20 μg/ml; the remaining compounds **1**–**7**, **10** had MBC from 100 to > 100 μg/ml.

The highest activity in 0,1 μg/ml on DR and MDR strains of MBT II and III was shown by hydrochloride, iodomethylate of O-*para*-toluoyl-β-(1-methylpiperazin-1-yl) propioamidoxime (**11**) (table 1).

The acute toxic effect of rifampicin, isoniazid, and compounds **8** and **11** (LD_{50}) was determined on white mice of both sexes weighing 17–23 g when administered subcutaneously. The toxicity of rifampicin SV is 267,6±7,2 mg/kg; of isoniazid – 62,5±12,8 mg/kg; toxicity of compounds **8** and **11**, respectively, – 325,0±17,8 and 1750,0±35,6 mg/kg [13].

Thus, O-benzoyl-β-(thiomorpholin-1-yl)propioamidoxime and hydrochloride, iodomethylate of O-*para*-toluoyl-β-(4-methylpiperazin-1-yl)propioamidoxime is much more active against the DS *M. tuberculosis* strains than rifampicin and isoniazid; whereas hydrochloride, iodomethylate of O-*para*-toluoyl-β-(4-methylpiperazin-1-yl)propioamidoxime is by 20 times more active against DR and MDR of *M. tuberculosis* strains than rifampicin SV and by 10 times more than isoniazid. O-Benzoyl-β-(thiomorpholin-1-yl)propioamidoxime is less toxic than rifampicin SV in 1,2 times and in 5,2 times less toxic than isoniazid;

hydrochloride, iodomethyl O-*para*-toluoyl-β-(4-methylpiperazin-1-yl)propioamidoxime is less toxic than rifampicin SV in 6,5 times and in 28 times less toxic than isoniazid. These data are protected by a patent and an innovative patent of the Republic of Kazakhstan [14, 15].

Based on the high priority requirements of increasing the effectiveness and safety of treatment in the development of new anti-tubercular drugs, it can be argued that O-benzoyl-β- (thiomorpholin-1-yl)propioamidoxime and hydrochloride, iodomethyl of O-*para*-toluoyl-β-(4-methyl piperazine-1-yl)propioamidoxime are competitive because they are less toxic and more active than the basic tuberculostatics used in practice – isoniazid and rifampicin.

EXPERIMENTAL CHEMICAL PART

IR spectra were taken on a NICOLET 5700 FT-IR instrument in KBr tablets. The ^{1}H NMR spectra are recorded in DMSO-d_6 on an Avance III 500 MHz spectrometer with an operating frequency for ^{1}H cores – 500 MHz with an internal standard hexamethyldisilane (HMDS; δ 0,05 ppm); measurements were carried out at a temperature of 25 °C.

The reaction was monitored using TLC on Sorbfil plates (CJSC Sorbpolymer) with a sorbent applied – a layer of CTX-1A silica gel, 5–17 μm granulation, UV indicator UV-254 using an ethanol:benzene mixture – 3:1 as eluent with the addition of concentrated ammonia. Solvents used in the synthesis, recrystallization of compounds and as eluents for TLC (EtOH, 2-PrOH, acetone, chloroform, diethyl ether) were prepared by standard methods. The preparation of compounds **1**–**10** is described in [12].

*Synthesis of hydrochloride, iodomethylate of O-para-toluoyl-β-(4-methylpiperazin-1-yl) propioamidoxime (**11**).* To 0,28 g (0,0015 mol) β-(4-methylpiperazin-1-yl)propioamidoxime in 30 ml of absolute ethanol at heating to 40 °C, 0,23 g (0,0015 mol) of *para*-toluoyl chloride was added and then 0,21 g (0,0015 mol) of methyl iodide was added. When evaporation of ethanol in half,

Table 2 – Physical and chemical data and IR and ^{1}H NMR spectra of hydrochloride, O-*para*-toluoyl-β-(4-methylpiperazin-1-yl)propioamidoxime iodomethyl (**11**)

# comp.	Output, %	M. p., °C	Found,% / Calculated, %		Formula	IR spectra ν, cm -1	Spectra ^{1}H NMR, M, ppm
			C	H			
11	69	222 (decomp.)	45,28 / 45,71	6,28 / 6,52	$C_{17}H_{28}ClIN_4O_2$	1643, 1595, 2853, 2925, 2959, 3012, 3139, 3292, 3396	2,60 t (7,0) (2H); 2,85 m (4H); 3,14 (3H); 3,24 (3H); 3,31 (3H); 3,69 t (7,0) (2H); 3,89 m (4H); 6,79 (2H); 7,50 d (10) (2H); 7,94 d (10) (2H); 10,90 (1H)

0,41 g (69%) of a white precipitate of hydrochloride, iodomethylate of O-*para*-toluoyl-β-(4-methylpiperazin-1-yl)propioamidoxime (**11**) were isolated with physical and chemical characteristics and spectral data indicated in table 2.

EXPERIMENTAL BIOLOGICAL PART

In vitro anti-tuberculosis activity was assessed as bactericidal [indicator – minimum inhibitory concentration (MIC, μg/ml)] on Shkolnikova liquid medium on 4 strains [sensitive *M. tuberculosis* H37Rv strain and on three wild strains of MTB: I – III].

The general method for determining in vitro bactericidal anti-tuberculosis activity (MBC) on Shkolnikova liquid medium. Into each tube with different concentrations of the studied drugs and into the control tube (nutrient medium without drug) 0,1 ml of mycobacterium tuberculosis suspension in 0,2 ml of physiological saline were seeded. The tubes were incubated at 37 °C for 10 days. After 10 days of the incubation in a liquid medium, the precipitates were centrifuged, washed with physiological saline, and the washings were seeded onto Levenshtein-Jensen solid medium without preparations. Accounting of the growth of cultures was carried out twice – after 1 month and after 2,5 months.

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Резюме

Л. А. Каюкова, К. Д. Пралиев, М. А. Оразбаева, Г. Т. Дюсембаева, А. Б. Узакова, Б. Т. Токсанбаева, В. Л. Биісмілда, Л. Т.Чингисова

β-(ТИОМОРФОЛИН-1-ИЛ)- ЖӘНЕ β-(4-МЕТИЛПИПЕРАЗИН-1-ИЛ)ПРОПИОАМИДОКСИМДЕРДІҢ О-АРОИЛДЕУ ӨНІМДЕРІ НЕГІЗДЕР МЕН ТҰЗДАРЫНЫҢ АРАСЫНАН ЖАҢА ТУБЕРКУЛЕЗГЕ ҚАРСЫ ЗАТТАРДЫҢ ІЗДЕЛІНУІ

Туберкулез - әлемдегі өлім мен аурудың негізгі себебі. 2017 жылы Қазақстанда туберкулезбен сырқаттанушылық деңгейі 52,2 және өлім-жітім 100 мың тұрғынға шаққанда 3,0 құрады. Әлемде туберкулезге қарсы жаңа дәрілік заттар ізделуде. Біз β-(тиоморфолин-1-ил) және β-(4-метилпипиразин-1-ил) пропиоамидоксимдердің О-ароилдеу өнімдерінің тұздары мен негіздерін синтездеп алдық. I*n vitro* туберкулезге қарсы скрининг β-аминопропиоамидоксим туындыларының *M.tuberculosis* ДС, ДТ және КДТ штамдарында улылығы рифампицин мен изониазидтен төменірек, белсенді бәсекеге түсе алатын қосылыстарды анықтады.

Түйін сөздер: туберкулез, О-ароил-β-(тиомор-фолин-1-ил)-пропиоамидоксимдер гидрохлоридтері мен негіздері, β-(4-метилпипиразин-1-ил)пропиоамидоксим гидрохлориді, йодметилаты, *M. tuberculosis* ДС, ДТ және КДТ штамдарына скрининг.

Резюме

Л. А. Каюкова, К. Д. Пралиев, М. А. Оразбаева, Г. Т. Дюсембаева, А. Б. Узакова, Б. Т. Токсанбаева, В. Л. Бисмилда, Л. Т.Чингисова

ПОИСК НОВЫХ ПРОТИВОТУБЕРКУЛЕЗНЫХ СРЕДСТВ СРЕДИ СОЛЕЙ И ОСНОВАНИЙ ПРОДУКТОВ О-АРОИЛИРОВАНИЯ β-(ТИОМОРФОЛИН-1-ИЛ)- И β-(4-МЕТИЛПИПЕРАЗИН-1-ИЛ)ПРОПИОАМИДОКСИМОВ

Туберкулез – ведущая причина смертности и заболеваемости в мире. В 2017 г. заболеваемость туберкулезом в Казахстане составляла 52,2 и смертность – 3,0 на 100 тыс. населения. В мире проводится поиск новых противотуберкулезных средств. Нами синтезированы соли и основания продуктов О-ароилирования β-(тиоморфолин-1-ил)- и β-(4-метилпиперазин-1-ил)пропиоамидоксимов. *In vitro* противотуберкулезный скрининг производных β-аминопропиоамидоксимов на ЛЧ, ЛУ и МЛУ штаммах *M. tuberculosis* выявил высокоактивные конкурентоспособные соединения, менее токсичные, чем рифампицин и изониазид.

Ключевые слова: туберкулез, гидрохлориды и основания О-ароил-β-(тиоморфолин-1-ил)-пропиоамидоксимов, гидрохллорид, йодметилат β-(4- метилпиперазин-1-ил)пропиоамидоксима, скрининг на ЛЧ, ЛУ и МЛУ штаммах *M. tuberculosis*.