RESEARCH ARTICLE

β-Aminopropioamidoximes derivatives as potential antitubercular agents against anthropozoonotic infections caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis*



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ABSTRACT

Background and Aim: Tuberculosis (TB) remains a significant global health challenge, with increasing incidences of drugsensitive (DS) and multidrug-resistant (MDR) TB. In addition, *Mycobacterium bovis*-induced zoonotic TB (zTB) presents treatment difficulties due to its resistance to pyrazinamide and the prolonged treatment duration required. This study aims to evaluate the antitubercular potential of β -aminopropioamidoxime derivatives against DS and MDR *M. tuberculosis* and *M. bovis* strains, and utilizing the SwissADME prognostic tool to predict the drug- and lead-likeness of the described compounds.

Materials and Methods: Six β -aminopropioamidoxime derivatives were synthesized through O-aroylation of amidoxime followed by dehydration to form 1,2,4-oxadiazoles. The compounds were tested *in vitro* against DS, MDR *M. tuberculosis,* and *M. bovis* using Sotton's liquid medium and subcultured on dense Lowenstein-Jensen medium. SwissADME was used to predict drug-likeness and pharmacokinetic properties.

Results: The derivatives exhibited significant antitubercular activity, with *in vitro* efficacy 5–100 times greater than rifampicin. 1,2,4-oxadiazoles with *para*-bromo and *meta*-chloro substituents demonstrated the highest activity against DS and MDR *M. tuberculosis*, while O-*para*-toluoyl- β -(morpholin-1-yl)propioamidoxime salts (hydrochloride, oxalate and citrate) were 10 times more active against *M. bovis*. SwissADME analysis confirmed favorable pharmacokinetic properties, including high gastrointestinal absorption and drug-likeness, with lead-likeness identified in four compounds.

Conclusion: The study presents β -aminopropioamidoxime derivatives as promising candidates for antitubercular therapy against both human and zTB. Their enhanced activity, oral bioavailability, and potential integration into new treatment regimens underscore their therapeutic relevance. Further *in vivo* studies are recommended to validate their efficacy and safety for clinical applications.

Keywords: antitubercular agents, *Mycobacterium bovis*, *Mycobacterium tuberculosis*, SwissADME, tuberculosis, β -aminopropioamidoximes

the COVID-19 pandemic [1, 2]. The emergence of *Mycobacterium tuberculosis* resistance has been

attributed to the prolonged use of standardized

regimens containing four first-line drugs, namely

vetworld.

INTRODUCTION

In recent years, there has been an increase in the global incidence of tuberculosis (TB) associated with several factors related to TB treatment during

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rifampicin, isoniazid, pyrazinamide, and ethambutol or streptomycin, for 6–9 months in treating drugsusceptible TB [3]. In addition, the treatment of drugresistant (DR), multidrug-resistant (MDR) TB is hindered by low drug efficacy, toxicity associated with treatment, and high costs, requiring a treatment duration of 20–24 months, primarily involving fluoroquinolones and second-line injectables, such as amikacin, capreomycin, or kanamycin [4–6]. The costs associated with MDR-TB treatment range from \$650 to \$8,266/patient. The costs associated with treating MDR-TB patients are significantly higher than those for DS-TB, costing patients a 20% of their annual household income [7].

Since the 1990s, several attempts have been made to determine treatment regimens that can overcome the shortcomings of existing regimens, including both new and repurposed drugs, in response to the urgent need for more effective treatment regimens for all types of DR TB. As of August 2023, 28 drugs for the treatment of TB have entered Phases I, II, and III trials [8].

The key change in the latest World Health Organization (WHO) recommendations is the addition and prioritization of a new all-oral 6-month (BPaLM) regimen for the treatment of DR TB [1].

TB is defined by 12 closely related members of the *Mycobacterium genus*, termed the *M. tuberculosis* complex, with *M. tuberculosis* and *M. bovis*, among others [9–12]. *M. bovis* exhibits almost complete resistance to pyrazinamide. Therefore, antibiotic regimens based on first-line anti-tubercular drugs (rifampicin, isoniazid, and ethambutol) should be considered. In addition, the duration of treatment for *M. bovis* is longer than that for *M. tuberculosis* (9 vs. 6 months) [13].

Of the 10 million newly reported cases of TB, with 1.6 million deaths, approximately 142,000 new cases and 12,500 deaths were attributed to *M. bovis* [14]. Termed "zoonotic TB," *M. bovis* cases in humans are likely underestimated due to a lack of reporting in endemic countries and limited laboratory capacity [15–17]. TB caused by *M. bovis* is well-defined by the World Organization for Animal Health, which comprises 179 countries [18].

The transmission routes of *M. bovis* remain poorly understood. It is generally assumed that transmission occurs through animals, mainly by aerosols. Humans may also transmit disease through the consumption of contaminated animal products such as raw milk, dairy products, and meat. In addition, contact with infected body fluids or tissues is another route of transmission that has a higher probability for slaughterhouse workers and hunters. In countries with TB surveillance programs, infected livestock is usually removed before developing clinical signs [19]. The impact of *M. bovis* epidemiology is growing, given the serious economic consequences: in international trade due to import bans on infected animals and animal products; in wildlife, species are driven to extinction; and in businesses, such as tourism, the impact is difficult to assess [20].

The misuse of anti-TB drugs leads to resistance in animals, raising global concerns regarding the potential transmission of the pathogen to humans [21].

Research under the framework of the "One Health" concept using computational methods can identify and type bacteria and propose new strategies to combat MDR bacteria in human and veterinary medicine. Sharing resources and increasing collaboration between public health and veterinary scientists will increase awareness of the "shared risk" of TB in humans and animals and, in resource-limited situations, maximize the use of existing infrastructure and reduce unnecessary duplication of efforts in disease control programs [22–24].

The new 6-month DR TB regimen (BPaLM or BPaLC) recommended by the WHO was based on the results of the TB-PRACTECAL trial, which was completed in 2022. The 6-month regimen in MDR-TB patients consists of the following new or repurposed drugs: bedaquiline, pretomanid, linezolid, and moxifloxacin (or clofazimine) [25].

Figure 1 illustrates the chemical structures of the drugs included in these regimens and their minimum bactericidal concentrations (MBCs) in the DS and MDR strains: bedaquiline [26, 27], pretomanid [28], linezolid [27, 29], moxifloxacin [27, 30], and clofazimine [27, 31].

In the early stages of drug development, the *in vitro* activity of pharmaceuticals and their chemical form are key factors. The bioavailability of possible dosage forms, water solubility, and stability are important. There is a risk of transformations during the non-clinical and clinical stages [32]. As a rule, the commercial appearance of the drugs that make up the TB-PRACTECAL regimen is in the form of salts: bedaquiline [33], linezolid [34], moxifloxacin [35], or as a suspension in an oil-wax base–clofazimine [36], or either in 0.5% methylcellulose or in cyclodextrin/ lecithin (CM2)–pretomanid [37].

Long-term treatment for TB results in side effects, the most common of which are as follows: acute or chronic inflammation of the tendons, hearing and vision loss, liver and kidney damage, neuropathy, and QT prolongation [38]. The rapid emergence of bacteria resistant to existing treatments necessitates the development of new molecules with antitubercular properties and optimization of existing anti-TB drugs [39].

Due to the relevance of TB, especially DR forms, in Kazakhstan [40] and the difficulties in treating MDR and XDR TB, our research group has been actively searching for novel anti-tubercular drugs.

The key compounds selected for synthetic modifications are β -aminopropioamidoximes. Sixmembered nitrogen-containing heterocycles with possible additional heteroatoms are used as β -amino



Figure 1: Preparations included in the shortened 6-month treatment regimen for patients with MDR/RR-TB or MDR/RR-TB with additional resistance to fluoroquinolones (pre-XDR-TB). MDR=Multidrug-resistant, RR-TB=Rifampicin-resistant tuberculosis, XDR-TB=Extensively drug-resistant tuberculosis [26–31].

substituents. The synthesis scheme consists of two stages: (1) the reaction of heterocyclic amines with acrylonitrile to form β -aminopropionitriles and (2) the production of β -aminopropioamidoximes in the reaction of β -aminopropionitriles with hydroxylamine (Scheme 1).

In the aroylation reactions of β -aminopropioamidoximes, only derivatives at the oxygen atom of the oxime fragment were obtained. The dehydration of O-aroyl derivatives leads to the formation of 3,5-disubstituted 1,2,4-oxadiazoles. The β -amino atom facilitates the generation of water-soluble salts. This is especially important in the development of antitubercular drugs, considering the duration of both DS and MDR TB treatment and the need to operate with a convenient water-soluble dosage form. In addition, the synthesis of the β -aminopropioamidoxime derivatives libraries was conducted based on the 3–4 stage scheme with good yields.

Figure 2 shows the most *in vitro* active molecules against DS *M. tuberculosis* H37Rv and MDR *M. tuberculosis* O-aroyl- β -aminopropioamidoximes and 3,5-disubstituted 1,2,4-oxadiazoles obtained before this work [41–43].

In accordance with the "One Health" idea [22–24], we conducted parallel *in vitro* screening of a group of β -aminopropioamidoximes derivatives on DS and MDR strains of *M. tuberculosis* and on *M. bovis* strains

The significance of this work is the development of a library of compounds from the β -aminopropioamidoxime class as potential antitubercular agents against anthropozoonotic infections caused by *M. tuberculosis* and *M. bovis*. Comparison of minimum inhibitory concentrations (MICs) of the β -aminopropioamidoxime derivatives we obtained with MICs of compounds from the different classes (bedaquiline – diarylquinoline; pretomanid – nitroimidazole; linezolid – oxazolidinone; moxifloxacin–fluoroquinolone; clofazimine–phenazine), which are components of the TB-PRACTECAL regimen for the treatment of MDR-TB, allows us to consider them competitive and have optimism for their further development. In the literature, there are no examples of systematic studies of new drugs within the One Health concept and in the area of β -aminopropioamidoximes as well.

The aim of this work was to demonstrate the potential of the β -aminopropioamidoxime derivatives developed by us in the treatment of human TB caused by DS and MDR *M. tuberculosis* and anthropozoonotic TB caused by *M. bovis*; in addition, based on available *in vitro* data, to determine their competitiveness with the components of the abbreviated BPaLM(C) regimen. Using the SwissADME prognostic product based on physicochemical data to evaluate drug- and lead-likeness of the group of compounds presented in the article.

Previously, we obtained 1,2,4-oxadiazoles (**3a**–c) derived from β -(piperidin-1-yl)propioamdoxime (**1a**) and studied their anti-TB properties on DS and MDR *M. tuberculosis* strains [43, 44]. Here, for the 1st time, we present the results of *in vitro M. bovis* testing. For the 1st time, we present the synthesis of a number of salts **4a**, c, d based on β -(morpholin-1-yl)propioamdoxime (**1b**) and the results of their testing on DS and MDR *M. tuberculosis* strains and on the *M. bovis* strain (Scheme 2 and Tables 1–4).

MATERIALS AND METHODS

Ethical approval

This study only involved laboratory testing of mycobacteria and did not include studies on animals or humans; therefore, no prior ethical approval was required.



Figure 2: The most *in vitro* active samples on DS *M. tuberculosis* H37Rv and MDR *M. tuberculosis* from the series of arylation products β -(4-phenylpiperazin-1-yl)- [41], β -(thiomorpholin-1-yl)- [42], β -(4-methylpiperazin-1-yl)- [42], β -(piperidin-1-yl)- [43] propioamidoximes and 1,2,4-oxadiazoles [43]. MTB=*M. tuberculosis*, DS=Drug sensitive, MDR=Multidrug-resistant.



Scheme 1: General method for the preparation of β -aminopropioamidoximes.



Scheme 2: Synthesis of 5-aryl-3-[β -(piperidin-1-yl)ethyl]-1,2,4-oxadiazoles (**3a**–**c**) and derivatives of O-*para*-toluoyl- β -(morpholin-1-yl)propioamidoxime: hydrochloride (**4a**), base form (**4b**), oxalate (**4c**), citrate (**4d**).

Table 1: Physicochemical data of O-*para*-toluoyl-β-(morpholin-1-yl) propioamidoxime (**4b**) and its pharmacologically acceptable salts: hydrochloride (**4a**), oxalate (**4c**), and citrate (**4d**).

Compound	Chemical form	Yield, %	mp, °C	R _f	Gross formula	Solubility in water
4a	Hydrochloride	83.2	146	0.43	C, H, CIN, O,	Highly soluble
4b	Base	93.0	82–6	0.49	C, H, N, O,	Insoluble
4c	Oxalate	62.0	151	0.50	C,,H,N,O,	Highly soluble
4d	Citrate	90	148	0.13	$C_{21}^{17}H_{2}^{23}SN_{3}O_{10}$	Highly soluble

mp=Melting point

Study period and location

The studies presented in the article were carried out in three research centers located in Almaty, Kazakhstan.

Study on the synthesis of the 5-aryl-3-[β -(piperidin-1-yl) ethyl]-1,2,4-oxadiazoles (**3a–c**) and O-aroyl- β -(morpholin-1-yl)propioamidoximes (**4a–d**) have been carried out

Table 2: Infrared (IR) spectra of O-para-toluoyl-β-(morpholin-1-yl) propioamidoxime (4b) and quaternary ammonium salts:
hydrochloride (4a), oxalate (4c), citrate (4d), KBr tablet, cm⁻¹.

Compound	Stretching and bending vibrations of bonds, cm ⁻¹ , KBr tablet									
	V _{C=0}	V _{C=N}	$\delta_{_{N-H}}$	V _{c=c}	V _{N-0}	v _{c-o}	V _{C-N}	v _{N(+)-H} (v _{Csp3-H})	$v_{_{N(-H)2}}$; $v_{_{COO-H}}$ and $v_{_{O-H}}$	
4a	1738	1662	1648	1605	1115	1282	1096	2546, 2666 (2855, 2923)	3215, 3309, and 3445	
4b	1731	1634	1612	1612	1113	1268	1097	(2825, 2869, 2928, 2968)	3132, 3364, and 3483	
4c	1752	1658	1613	1613	1175	1263, 1280	1079	2522, 2612, 2684; (2830, 2903)	3202, 3324, and 3435	
4d	1772	1653	1600	1600	1129	1232	1100	2640, 2730 (2894, 2962)	3121,3525	

KBr=Potassium bromide

Table 3: ¹H NMR spectra of O-*para*-toluoyl- β -(morpholin-1-yl) propioamidoxime (**4b**) and quaternary ammonium salts: hydrochloride (**4a**), oxalate (**4c**), and citrate (**4d**).

Compound	Chemical shifts, δ, ppm (J, Hz)*											
	O (CH ₂) ₂	N (CH2)2 (4b); N(+)(CH ₂) ₂ (4a, 4c, 4d)	α-CH ₂	β-CH ₂	NH ₂	N(+) H	Para-CH ₃	C_6H_4				
4a	3.42t	3.93 m	2.78t	3.20 m	6.86	11.61	2.37	7.31d (8.1);				
	(8.1)		(8.4)	(8.4)				8.01d (8.1)				
4b	3.53 m	2.32 m	2.26t	2.54t	6.56	-	2.28	7.25d (8.0);				
			(7.0)	(7.0)				7.96d (8.0)				
4c	3.04 m	3.75 m	2.55t	3.16t	6.71	11.58	2.32	7.27d (8.0);				
			(7.0)	(7.0)				7.97d (8.0)				
4d	2.58 m	2.64 m	2.38t	2.80t	6.68	12.05	2.32	7.25d (8.0);				
			(7.0)	(7.0)				7.96d (8.0)				

*Proton signals of carboxylate anions: 9.38 ppm (HOOCCOO⁻) (4c); 2.54 (OH), 3.61 m (CH,),, 8.83 br (COOH) (4d). ¹H NMR=Proton nuclear magnetic resonance

Table 4: *In vitro* anti-tubercular activity of the β -aminopropioamidoxime derivatives **3ac**, **4a**, **4c**, and **4d** against DS (H37Rv), MDR MTB, and *M. bovis* strains^{*}.

Compound	3a	3b	3c	4a	4c	4d	Rifampicin
MBC DS MTB H37Rv, μg/mL	1	0.01	0.01	4**	0.1	0.1	1 (20)*
MBC MDR MTB, μg/mL	2	0.01	0.01	-	0.1	0.1	2
MBC <i>M. bovis,</i> μg/mL	1	1	1	0.1	0.1	0.1	1

*The MBC value of **4a** in the DS MTB H37Rv strain was 4 µg/mL; under these experimental conditions rifampicin MBC value was 20 µg/mL. MDR=Multidrug-resistant, MTB=*Mycobacterium tuberculosis*, MBC=Minimum bactericidal concentration, *M. bovis=Mycobacterium bovis*, DS=Drug-sensitive

since the January to November 2002 for the first group of compounds and since the Januare to December 2018 for the second group of compounds in the Laboratory of Chemistry of Synthetic and Natural Drugs of JSC A.B. Bekturov Institute of Chemical Sciences (http://ihn. kz/). During the same time periods, biological screening for DS and MDR strains of M. tuberculosis was carried out for the corresponding groups of compounds in the National Reference Bacteriological Laboratory of the National Scientific Center of Phthisiopulmonology (https://www.nncf.kz/). Screening on M. bovis strains was performed in March to June 2024 at LLP Scientific and Production Center Biovet, Almaty, Kazakhstan (https://www.emis.com/php/company-profile/KZ/ Nauchno-Proizvodstvennyi Tsentr Biovet), which works in the field of scientific research in the veterinary sector.

Synthesis of β-aminopropioamidoximes derivatives

This research mainly involved the synthesis of two structural groups of β -aminopropioamidoxime derivatives: **3a–c** and **4a–d**. In essence, the starting compounds are β -aminopropioamidoximes **1a** and **1b** [β -amino group: piperidin-1-yl (**1a**); morpholin-1-yl (**1b**)]. O-aroyl derivatives of amidoxime **2a–c** and **4a–d** were formed during the interaction of β -aminopropioamidoximes **1a** and **1b** with the substituted benzoic acid chlorides. 5-Aryl-3-[β -(piperidin-1-yl)ethyl]-1,2,4-oxadiazoles (**3a–c**) were obtained following dehydration of β -piperidine derivatives **2a–c**.

The target compounds **3a–c** and **4a–d** were purified through recrystallization and characterized by physicochemical (yield, %, melting point [mp], °C, and mobility indicator in thin-layer chromatography $[R_r]$) and spectral data (infrared [IR] spectroscopy and proton nuclear magnetic resonance [¹H NMR] spectroscopy).

The IR spectra of compounds **3a–3c** and **4a–d** were obtained using an FSM2201 spectrometer (Infraspek, St. Petersburg, Russia) with KBr tablets. ¹H spectra were recorded on a Bruker Avance III 500 MHz NMR spectrometer (Bruker, BioSpin GmbH, Rheinstetten, Germany). The residual non-deuterated solvent deuterated dimethyl sulfoxide (2.50 ppm) was used as a reference. Elemental analysis was performed using a CE440 elemental analyzer (Exeter Analytical, Inc., Shanghai, China). Mps were determined in glass capillaries using a Stuart melting point apparatus SMP30 (Bibby Scientific Ltd., Stone, Staffordshire,

UK). The completion of the reaction and the purity of the synthesized product were monitored using thinlayer chromatography (TLC) plates of the Sorbfil brand (Sorbpolymer, Krasnodar, Russia), coated with silica gel CTX-1A, grain size 5–17 μ m, and containing the UV-254 indicator. The benzene–ethanol mixture (1:3) was used. Sigma-Aldridge reagents were used without purification. The solvents for synthesis, recrystallization, and TLC analysis (ethanol, dimethylformamide [DMF], isopropanol [i-PrOH], chloroform, benzene, acetone, and ethyl acetate [EtOAc]) were purified using known methods.

5-Aryl-3-[β-(piperidin-1-yl)ethyl]-1,2,4-oxadiazoles (**3a**–c) were obtained through O-aroyl-(2-piperidin-1-yl) propioamidoximes (**2a**–c) dehydration in DMF at 70°C (Scheme 2), as previously described by Kayukova *et al.* [44, 45] and β-(morpholin-1-yl)propioamidoxime (**1b**). Morpholine (15.00 g; 0.172 mol) in 50 mL of absolute ethanol was placed in a 250 mL three-horned flask equipped with a refrigerator, stirrer, and drip funnel. A freshly distilled acrylonitrile (9.12 g; 0.172 mol) in 20 mL of absolute ethanol was added dropwise at mixing and cooling up to 15°C with cold water. The reaction mixture was mixed at room temperature (RT; 20°C–22°C) for 20 h; β-(morpholin-1-yl)propionitrile has a TLC stain at *R*_e 0.80.

Hydroxylamine hydrochloride (11.95 g; 0.172 mol) was added to the reaction mixture. Everything was mixed until a homogeneous state was achieved. The sodium ethylate (Na⁺C₂H₅O⁻) solution obtained from 3.95 g (0.172 mol) of metallic Na and 50 mL of absolute ethanol was added dropwise at 10°C while stirring. The reaction mixture was mixed at RT for 3 days; a precipitate, a mixture of β-(morpholin-1-yl)propioamidoxime (**1b**) and sodium chloride (NaCl), was filtered. Precipitation of **1b** gathered from evaporated alcohol filtrate and the extraction of a mixture of amidoxime **1b** and NaCl in the Soxhlet's apparatus were crystallized from i-PrOH with β-(morpholin-1-yl)propioamidoxime (**1b**). This yields 9.65 g (66%), mp 136 °C, $R_{\rm f}$ 0.29. Anal. Calcd for $C_{\rm 7}$ H₁₅N₃O₂ (173.12), %: C, 48.54; H, 8.73. Found: C, 48.06; H, 8.95.

Hydrochloride (4a). *para*-Toluoylchloride (0.32 g; 0.0021 mol) in 5 mL of dried EtOAc was added with stirring to 0.36 g (0.0021 mol) β-(morpholin-1-yl) propioamidoxime (1b) in 30 mL of dried EtOAc. The reaction mixture was stirred at RT for 6 h. The precipitate of technical 4a was filtered and recrystallized from i-PrOH. Hydrochloride 4a (0.32 g; 83.2%), mp 124 °C, R_f 0.46 was obtained. Anal. Calcd for $C_{15}H_{22}ClN_3O_3(327.81)$, %: C, 54.96; H, 6.76; Cl, 10.82. Found, %: 55.35; H, 6.95; Cl, 9.78.

O - p a r a - Toluoyl- β -(morpholin-1-yl) propioamidoxime (**4b**). K₂CO₃ (0.25 g; 0.0018 mol) was added to 0.32 g (0.0018 mol) of O-para-toluoyl- β -(morpholin-1-yl)propioamidoxime hydrochloride (**4a**) in 10 mL of distilled water. Precipitate **4b** was formed after stirring at RT for 1 h; its recrystallization from i-PrOH yielded 24.18 g (93.0%) O-*para*-toluoyl-β-(morpholin-1-yl)propioamidoxime (**4b**) with mp 115°C, $R_{\rm f}$ 0.76. Anal. Calcd for C₁₅H₂₁N₃O₃ (291.35), %: C, 61.84; H, 7.27. Found, %: C, 62.20; H, 7.55.

Oxalate (4c). O-*para*-Toluoyl- β -(morpholin-1-yl) propioamidoxime (4b) (0.5 g; 0.0017 mol) and oxalic acid (0.15 g; 0.0017 mol) in 50 mL of acetone were refluxed under TLC control for 5 h. The precipitated salt was filtered, dried, and recrystallized from ethanol; oxalate 4c yield was 0.4 g (62%), mp 140°C, R_f 0.60. Anal. Calcd for $C_{17}H_{22}N_3O_7$ (380.15), %: C, 53.68; H, 5.83. Found: C, 53.37; H, 5.95.

Citrate (4d). O-*para*-Toluoyl- β -(morpholin-1-yl) propioamidoxime (4b) (1 g; 0.0034 mol) and citric acid (0.65 g; 0.0034 mol) in 100 mL of acetone were stirred under TLC control for 30 min; a white matter precipitate was formed throughout the reaction mixture. The precipitate 4d was filtered by adding 10 mL of acetone. Citrate 4d (1.48 g; 90%), mp 54°C, R_f 0.75, was obtained following recrystallization from ethanol. Calcd for $C_{21}H_{29}N_3O_{10}$ (483.47), %: C, 52.17; H, 6.05. Found, %: C, 52.57; H, 6.38.

All compounds presented here are water-soluble, which is necessary for the oral administration of antitubercular drugs.

In vitro bactericidal anti-tubercular activity and MBC in Sotton's liquid medium

The compounds presented herein were selected based on the MBC values of < 100 μ g/mL and the technological availability of samples from an array that had undergone *in vitro* screening against DS *M. tuberculosis* H37Rv and MDR *M. tuberculosis*. The selected samples were candidates for *in vitro M. bovis* screening.

The strains tested

DS *M. tuberculosis* H37Rv is a museum strain obtained from the National Scientific Center of Phthisiopulmonology strains collection, a wild strain of *M. tuberculosis* isolated from a patient in the National Scientific Center of Phthisiopulmonology clinic, typed as MDR, resistant to rifampicin and isoniazid; *M. bovis* is a museum strain received from the strain collection stored at JSC Kazakh Research Veterinary Institute.

Nutrient mediums

In vitro screening was performed on nutrient mediums: Sotton's liquid medium (HiMedia M1276-500G Sauton's Fluid Medium Base) and Lowenstein-Jensen dense medium prepared from the base (TB-Medium Base according to Lowenstein-Jensen, Sigma Aldrich, Catalog no. 63237) was obtained from JSCKelun-Kazpharm.

Standard used

Rifampicin (AppliChem, Darmstadt, Germany) was used as a standard for comparison with the tested drugs because it is commonly used in TB treatment.

The studied drug concentration ranged from 100 to 0.01 μ g/mL based on 10-fold dilutions. For each

drug concentration, three test tubes were used, and the experiment was conducted in two stages with two series. A mycobacterial culture (14–21 days) grown on a dense egg medium was removed from the slants under sterile conditions using a platinum spatula, ground in a test tube, and suspended in a 0.9% NaCl solution (physiological solution; TOOKelun-Kazpharm). After settling the large particles of the culture, the test tube was kept at RT for 20 min. Suspended particles of the generous size of the culture were allowed to settle, and the test tube was kept at RT for 20 min.

The bacterial suspension was collected using a pipette and transferred to another test tube. Muddiness, corresponding to the 5th standard, was achieved by adding the physiological solution to the test tube; 1 mL of suspension corresponded to the 5th standard of optical density containing 5×10^8 microbial bodies.

A 0.1 mL suspension of *M. tuberculosis* in 0.2 mL of physiological solution was added to each test tube with different concentrations of the studied drugs. The control tube contained a nutrient medium without the drug. The tubes were incubated at 37°C for 10 days in Sotton's liquid medium, following which the sediments were centrifuged and washed with physiological solution, and the washings were inoculated on the dense Lowenstein-Jensen medium. The growth of the colonies was recorded after 1 and 2.5 months [46].

Rifampicin was added to the *M. tuberculosis* solution at a concentration equal to that of the test drugs and incubated for the same incubation period as the test drugs in the other tubes.

The results of the cultural studies were recorded after 21–28 days and 2.5 months of cultivation in a thermostat at 37 $^{\circ}$ C according to the following scheme:

0: No MBT colonies on the shoal;

+: Single, up to 20 MBT colonies;

++: 20-100 MBT colonies;

+++: > 100 MBT colonies.

Table 4 lists the bactericidal concentrations of compounds **3a–c**, **4a**, **4c**, and **4d**, corresponding to values of 0 from the given designations.

Software for in silico SwissADME prediction

ChemDraw Ultra Software V.12.0.2 and Spartan'14V 1.1.4 (http://www.swissadme.ch) [47] were used for in silico SwissADME prediction.

RESULTS

Physicochemical data and structure

1,2,4-Oxadiazoles (3a-c) were obtained by dehydration in DMF at 70°C of O-aroyl derivatives (2a-c)(Scheme 2) [44]. β -(Morpholin-1-yl)propioamidoxime (**1b**) was prepared using a one-pot method, the first stage of which was the reaction of morpholine with acrylonitrile in ethanol to form β -(morpholin-1-yl) propionitrile followed by the reaction with hydroxylamine in this same reactionary environment. Amidoxime **1b** was isolated with a yield of 66% [45]. Interaction of amidoxime (**1b**) and *para*toluoyl chloride in EtOAc formed hydrochloride (**4a**) with a yield of 83.2%. O-*para*-toluoyl- β morpholinopropioamidoxime oxalate (**4c**) was obtained by the interaction of equivalent amounts of O-*para*-toluoyl- β -morpholinopropioamidoxime (**4b**) and oxalic acid in boiling acetone with a yield of 62%. Using the same method, when O-*para*-toluoyl- β -morpholinopropioamidoxime (**4b**) was reacted with citric acid, citrate (**4d**) was formed with a yield of 90% (Table 1).

The IR spectra of compounds **4a**, **4c**, and **4d** showed evidence of ammonium compound formation: the stretching vibration double bands $v_{c=0}$ and $v_{c=N}$ shifted to higher wave numbers. In addition, in the Fourier transform IR spectra of the salts **4a**, **4c**, and **4d**, a set of stretching vibration bands of the ammonium bonds appeared in the region 2522–2730 cm⁻¹ (Table 2).

As a rule, in the ¹H NMR spectra, the formation of a quaternary charged nitrogen atom in **4a,4c**, and **4d** shifts the proton signals of the α - and β -methylene groups and the NH₂ groups' proton signals to lower fields. In ¹H NMR spectra of **4c** and **4d**, the signals of the carboxylate anions protons appeared (Table 3).

In ¹H NMR spectra of **4c** and **4d**, the signals of the carboxylate anions protons appeared.

In vitro antitubercular screening of the β -aminopropioamidoximes derivatives 3a–c, 4a, 4c, and 4d against *M. tuberculosis* and *M. bovis*

As shown by *in vitro* screening (Table 4), compounds **3ac**, **4a**, **4c**, and **4d** demonstrated significant bactericidal activity, inhibiting the growth of DS and MDR *M. tuberculosis* and *M. bovis* strains. The MBC of rifampicin against *M. tuberculosis* H37Rv strain was 20 and 1 μ g/mL, against the MDR wild strain of *M. tuberculosis* was 2 mkg/mL, and against *M. bovis* was 1 μ g/mL.

The comparison of the MBC values of the β -aminopropioamidoxime derivatives **3a–c**, **4a**, **4c**, and **4d** with the MBC values of rifampicin showed that the compounds under study have the following properties:

- 1,2,4-Oxadiazoles 3a-c have MBC values equal to rifampicin - 1 μg/mL against *M. bovis* and 2 μg/mL against MDR *M. tuberculosis* (3a).
- O-para-Toluoyl-β-morpholinopropioamidoxime salts: hydrochloride (4a), oxalate (4c) and citrate (4d) have MBC values 5–10 times lower (4 and 0.1 µg/mL) than that of rifampicin (20 and 1.0 µg/mL) against DS M. tuberculosis H37Rv strain. Oxalate (4c) and citrate (4d) show MBC (0.1 µg/mL) against MDR *M. tuberculosis* strain, что в 20 times lower than rifampicin (2 µg/mL). Also, the entire set of salts (4a, 4c, and 4d) had a 10-fold lower MBC (0.1 µg/mL) against *M. bovis* strain than rifampicin (1µg/mL).
- 1,2,4-Oxadiazoles **3b** and **3c** against the DS strain of *M. tuberculosis H37Rv* show MBC values 100 times lower than those of rifampicin MBC (0.01 μg/mL and 1 μg/mL, respectively).

At the same time, these compounds **3b** and **3c** against MDR *M. tuberculosis* strain show MBC values 200 times lower than those of rifampicin (0.01 µg/mL and 2 µg/mL, respectively).

However, when switching from *in vitro* screening of 1,2,4-oxadiazoles **3b** and **3c** against *M. tuberculosis* to *in vitro* screening against *M. bovis*, there is no parallelism in maintaining the bactericidal effect. Their MBC against *M. bovis* decreased from 0.01 to $1 \mu g/mL$.

In silico absorption, distribution, metabolism, and excretion (ADME)/pharmacokinetic predictions

Based on bioavailability radar, initial assessment displayed varying degrees of drug-likeness of β -aminopropioamidoximes derivatives **3a–c** and **4a–d** (Figure 3).

The graphical images of the molecular radars and ADME drug-likeness parameters were obtained based on their molecular input line SMILES (simplified

Table 5: SMILES for calculating ADME parameters.

Compound	SMILES
3a	CC1=CC=C (C=C1) C1=NC (CCN2CCCCC2)=NO1
3b	BrC1=CC=C (C=C1) C1=NC (CCN2CCCCC2)=NO1
3c	CIC1=CC=CC(=C1) C1=NC (CCN2CCCCC2)=NO1
4a	[CI-].[H][N+]1(CC\C (N)=N\OC(=O) C2=CC=C (C)
	C=C2) CCOCC1
4b	CC1=CC=C (C=C1) C(=O) O\N=C(/N) CCN1CCOCC1
4c	OC(=O) C([O-])=O.[H][N+]1(CC\C (N)=N\OC(=O)
	C2=CC=C (C) C=C2) CCOCC1
4d	OC(=O) CC (O)(CC([O-])=O) C (O)=O.[H][N+]1(CC\C
	(N)=N\OC(=O) C2=CC=C (C) C=C2) CCOCC1

SMILES=Simplified molecular input line entry system, ADME=Absorption, distribution, metabolism, and excretion

molecular input line entry system) (Tables 5 and 6), which were formed after entering into the SwissADME system the structures of molecules depicted in the ChemDraw program.

Six physicochemical properties were considered: lipophilicity, size, polarity, solubility, flexibility, and saturation, displayed on each axis using optimal descriptor values [47].

A molecule is recognized as drug-like if its Radar plot falls within the pink area [48]. When examining the Bioavailability Radar plots of compounds **3a–3c** and **4a–4d**, we see that the first 5 compounds **3a–3c**, **4a**, and **4b** fall within the pink hexagon; to a first approximation, this allows us to consider them drug-like. The Radar plots of compounds **4c** and **4d** indicate their lower bioavailability due to the polarity of compound **4c** and the polarity and flexibility of compound **4d**.

ADME parameters can help to make informed decisions on the selection of promising drug-like compounds from the beta-aminopropioamidoxime derivatives studied at the *in vitro* stage. These parameters evaluate drug-like candidates that can be introduced into a biological system, taking into account their similarity to drugs (Table 6) [49, 50].

To assess drug similarity and determine whether the test compound will be orally active in humans, the Lipinski rule of five (Ro5) [51] is proposed. It is assumed that a molecule or an inhibitor can be orally absorbed/ active if two (2) or more of these starting points satisfy the following conditions: Molecular weight (MW) of molecule <500, octanol/water partition coefficient (iLOGP) \leq 5, number of hydrogen bond acceptors (nHBA) \leq 10, and number of hydrogen bond donors (nHBD) \leq 5.

Table	e 6: ADME and	drug-likeness	parameters of	f β-amino	propioamic	doxime derivatives.	
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Indicator	3a	3b	Зc	4a	4b	4c	4d
Physicochemical propertie	S						
MW (g/mol)	271.36	336.23	291.78	327.61	291.35	381.36	483.47
nHBA	4	4	4	4	5	8	11
nHBD	0	0	0	2	1	3	5
TPSA(Å)	42.16	42.16	42.16	78.356	77.15	155.76	213.31
Lipophilicity							
ilogp	3.70	3.81	3.71	0.00	2.62	2.26	1.93
Water solubility							
Class	Soluble	Moderately soluble	Moderately soluble	Soluble	Soluble	Very soluble	Very soluble
Pharmacokinetics						·	·
GI absorption	High	High	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	No	No	No	No
Drug-likeness							
Lipinski	Yes; 0 V	Yes; 0 V	Yes; 0 V	Yes; 0 V	Yes; 0 V	Yes; 0 V	Yes; 1 V N or O >10
Bioavailability Score	0.55	0.55	0.55	0.55	0.55	0.55	0.11
Medicinal chemistry							
Lead likeness	Yes	No; 1V: XLOGP3	No; 1: XLOGP3 >3.5	Yes	Yes	Yes	No; 1V:
		>3.5					Rotors >7
Synthetic accessibility	3.07	2.99	2.97	3.10	3.03	3.35	4.36

V=Violation, MW=(g/mol) molecular weight, nHBA=Number of hydrogen bond acceptor, nHBD=Number of hydrogen bond donor, TPSA=Topological polar surface area, iLOGP-Octanol/water partition coefficient, SA=Synthetic accessibility, GI=Absorption gastrointestinal absorption, BBB=Permeant blood–brain barrier permeant, ADME=Absorption, distribution, metabolism, and excretion

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From the meanings of drug-likeness properties shown in Table 5, it was observed that **3a–3c** and **4a–4d** molecules have zero violations of Lipinski's rule. Thus, the MWs of all compounds <500 g/mol), iLOGP is in the range of $0.00 \div 3.81$; the number nHBA ≤ 10 except for compound **4d**; and the numbers nHBD are in the range of $0 \div 5$. Thus, based on the Physicochemical Properties and Lipophilicity given in Table 5, it can

be concluded that the compounds under study are drug-like.

Drug-likeness parameters are related to aqueous solubility, and intestinal permeability determines the first step of oral bioavailability [47].

Topological polar surface area (TPSA) values reflect the area of the polar molecule and are increased as the number of polar groups in the molecule increases; functional groups containing nitrogen and oxygen atoms contribute to polarity and increase the TPSA value for a drug [52]. This feature is observed in the raw data of compounds **3a–3c** and **4a–4d**; the TPSA values vary from 42.16 to 213.31 Å².

Drugs with higher TPSA values are less lipidsoluble and will, in general, be absorbed less extensively and more slowly and will distribute less extensively than drugs with lower TPSA values. TPSA values are related to gastrointestinal absorption and blood-brain barrier (BBB) crossing. All compounds have a high gastrointestinal absorption (HI) value – "High." Different "blood–Brain Barrier Permeant" values for a series of 1,2,4-oxadiazoles (**3a–c**) – "High" and for a series of **4a–d** - "No" demonstrates how increasing polarity in the second row reduces the ability to penetrate the BBB.

Bioavailability defines the ability of a drug molecule to access cells by crossing cell membranes. A bioavailability score, BS, is described as the probability that a compound will have >10% bioavailability in rats. It defines four classes of compounds with probabilities to cross cell membranes of 11%, 17%, 56%, or 85%, the TPSA values of which fall into 4 different areas: TPSA is >150 Å² (11%); TPSA is between 75 and 150 Å² (56%); and TPSA is <75 Å² (85%). The remaining compounds have a BS equal to 0.55 if they pass the rule-of-five and 0.17 if they fail [53].

The BS of compounds **3a–c** and **4a–d** are represented by two values: 0.55 for compounds **3a–c** and **4a–c** and 0.11 for compound **4d**. The latter compound had the largest value of TPSA 213.31 Å² and one deviation from Lipinski's rule, while the TPSA area of the first group of compounds is 42.16 ÷ 155.76 Å². These data are in approximate agreement with the aforementioned limitations [53], and the most polar compound should have the lowest bioavailability.

In the studied series, lead-likeness was established for four out of seven compounds; this indicator is inherent in 5-toluene-3- $[\beta$ -(piperidin-1-yl)ethyl]-1,2,4-oxadiazole and O-toluoyl- β -(morpholin-1-yl) propioamidoxime hydrochloride, base, and oxalate.

Derivatives of β -aminopropioamidoximes have acceptable synthetic availability in the range of 2.97÷4.36, which is closer to the easy level under the condition «1» – easily available and «10» – difficult to access.

DISCUSSION

According to administrative data of the healthcare system of the Republic of Kazakhstan on

the epidemiology and mortality of tuberculosis in Kazakhstan, of the 149,122 patients with TB, from 2014 to 2019, TB incidence declined from 227 to 15.2 per 100,000 individuals, while all-cause mortality increased from 8.4 to 15.2 per 100,000 individuals [54]. The following comorbid conditions that complicate treatment, such as HIV [55], DM [56], stroke [57], chronic kidney disease (CKD) [58], and liver cirrhosis [59] were present in patients with TB.

Despite WHO recommendations to treat TB in an outpatient setting, Kazakhstan still has a high hospitalization rate at TB centers [60]. The latter circumstance led to an increased risk of in-hospital transmission of drug-resistant TB and is the decisive reason of the mortality rate during 2014–2019.

In Kazakhstan, the prevalence of DR-TB remains on average at the level of 45%–49% of all registered TB cases, which was the basis for its inclusion in the list of countries in Eastern Europe and Central Asia with a high burden of DR-TB [61]. Kazakhstan recognizes the importance of achieving the goal of "Ending the TB Epidemic by 2030" set out in the Sustainable Development Goals, the Political Declaration to Combat Tuberculosis, endorsed by the UN General Assembly, and the World Health Organization's Stop TB Strategy [62].

Treatment of patients with active tuberculosis in Kazakhstan is carried out with anti-tuberculosis drugs within the framework of the guaranteed volume of free medical care. Within the framework of the implementation of pilot projects, all-oral injection-free, shortened treatment regimens for MDR TB with new anti-TB drugs are used:

1) Levofloxacin (Lfx), bedaquiline (Bdq), linezolid (Lzd), clofazimine (Cfz), and delamanid (Dlm). 2) Bedaquiline (Bdq), linezolid (Lzd), levofloxacin (Lfx), clofazimine (Cfz), and cycloserine (Cs) [pyrazinamide (Z)]. The total course of treatment is 6–9 months with daily administration of drugs. The duration of use of bedaquiline and delamanid is 6 months. Clinically-relevant adverse events of special interest were uncommon. All regimens demonstrated excellent safety and effectiveness, expanding the potential treatment options for patients, providers, and programs [63, 64].

The problem of Kazakhstan's pharmaceutical science, which is probably a problem for many middleincome countries, is that the local pharmaceutical industry produces mainly generics, while original products are imported from abroad. However, new pharmaceuticals are being developed by scientists in Kazakhstan, regardless of the difficult and lengthy path of introduction into medical practice. An example of this are our works [41–43].

In addition, within the framework of the Development of New Anti-Infective Drugs program (JSC Scientific Center for Anti-Infective Drugs, Almaty, Kazakhstan) for many years, it was created as an original anti-tuberculosis drug FS-1 which is an ionic nanostructured complex of carbohydrates of proteins and polypeptides, iodine, and halides of alkaline and alkaline earth elements. FS-1 is highly effective against various Gram-positive and Gram-negative bacteria, including those resistant to antibiotics. MIC FS-1 varied over a wide range from 0.02 to 0.3 mg/mL. The main mechanism of action of FS-1 consists in membranolytic activity [65]. At present, this drug has passed the phase 3 of clinical trials [66].

Aligned with the "One Health" approach [22–24], this study identified a series of β -aminopropioamidoxime derivatives as highly active, watersoluble, and technologically advanced candidates for antitubercular therapy. These compounds demonstrated remarkable activity, showing 5–10 times higher efficacy against human strains of *M. tuberculosis* H37Rv, 20 times greater effectiveness against MDR *M. tuberculosis* strains, and a 10-fold improvement against zoonotic infections caused by *M. bovis*.

A comparative analysis of MBCs, synthetic pathways, and physicochemical properties of the β -aminopropioamidoximes derivatives with current drugs in the short oral regimen for MDR TB (including bedaguiline, pretomanid, linezolid, moxifloxacin, and clofazimine) highlighted the distinct advantages of these new derivatives. The notable in vitro activity, solubility, simplified synthesis process, water and overcoming drug resistance, along with the significant adverse effects associated with existing anti-tuberculosis drugs, underscores the potential of β -aminopropioamidoximes derivatives as a promising new class of anti-TB agents.

The simultaneous screening of β -aminopropioamidoximes derivatives against DS and MDR human *M*. *tuberculosis*, as well as zoonotic *M*. *bovis* strains, offers a holistic strategy for addressing critical health challenges and advancing the eradication of TB infections through new, highly potent therapeutic options.

The anti-tubercular screening results revealed that all tested β -aminopropioamidoximes derivatives, except compound **3a**, demonstrated MBC values 5–100 times lower than rifampicin against DS and MDR strains of *M. tuberculosis*. Notably, compound **3a** exhibited activity comparable to rifampicin. Against *M. bovis*, compounds **3a–c** showed equivalent activity to rifampicin, whereas salts **4a**, **4c**, and **4d** demonstrated tenfold higher efficacy against all tested strains. Compounds **3b** and **3c**, being 100 times more potent than rifampicin, exhibit promising potential for clinical applications in treating DS and MDR human TB. The introduction of new active molecules for managing anthropozoonotic infections caused by human DS and MDR *M. tuberculosis* and *M. bovis* strains is therefore requested.

In silico ADME analysis affirmed the strong druglikeness of β -aminopropioamidoxime derivatives, with favorable pharmacokinetic characteristics, including high solubility and efficient gastrointestinal absorption. The analysis indicated the ability of 1,2,4-oxadiazoles to permeate the BBB and the lack of this property in certain salts and the base of O-toluoyl- β -(morpholin-1-yl)propioamidoxime.

The bioavailability values revealed the varied capacity of β -aminopropioamidoxime derivatives to traverse cell membranes, with O-toluoyl- β -(morpholin-1-yl)propioamidoxime citrate showing the lowest bioavailability due to its high polarity. Among the tested compounds, four demonstrated lead-likeness, and the derivatives of β -aminopropioamidoximes showed acceptable synthetic accessibility.

Considering the high costs and prolonged duration of current MDR TB treatments, along with the simplified synthesis of β -aminopropioamidoximes derivatives achieving quantitative yields at every stage, this study emphasizes the potential of these compounds as a novel group of anti-tubercular agents. However, a major limitation in developing new anti-tubercular drugs is the financial and temporal burden of extensive preclinical and clinical trials, which is particularly challenging for countries with constrained research budgets, such as Kazakhstan.

CONCLUSION

This study introduces a novel library of β-aminopropioamidoxime derivatives with promising antitubercular properties against both human and zoonotic TB (zTB) strains. The synthesized compounds exhibited remarkable in vitro efficacy, demonstrating 5-100 times greater activity than rifampicin against DS and MDR M. tuberculosis and M. bovis strains. Notably, 1,2,4-oxadiazoles with para-bromo and meta-chloro substituents on the phenyl ring exhibited the highest potency against M. tuberculosis, while O-para-toluoyl- β -(morpholin-1-yl)propioamidoxime salts showed significant activity against M. bovis. The in silico ADME analysis supported the drug-likeness of these compounds, highlighting their favorable pharmacokinetic profiles, including good solubility, high gastrointestinal absorption, and selective BBB permeability.

The study successfully integrated a "One Health" approach by evaluating the compounds against both human and zTB strains, enhancing the potential for broad-spectrum therapeutic applications. The combined *in vitro* and *in silico* analyses provided a robust assessment of the compounds' efficacy, bioavailability, and drug-likeness, offering a strong foundation for further development. In addition, the compounds' simple synthetic routes, high yields, and water solubility align well with the requirements for practical drug development.

Despite the promising *in vitro* results, the study is limited by the absence of *in vivo* evaluations, which

are critical to establishing the therapeutic efficacy and safety of β -aminopropioamidoxime derivatives in living organisms. In addition, potential toxicity and long-term safety profiles were not assessed, necessitating comprehensive preclinical studies. The study's findings are also confined to laboratory conditions, which may not fully replicate clinical settings.

Future research should focus on *in vivo* studies to validate the antitubercular efficacy of β -aminopropioamidoxime derivatives in animal models. Pharmacokinetic and toxicological assessments are essential to ensure safety and therapeutic viability. The compounds showing selective BBB permeability could also be explored for treating central nervous system TB infections. In addition, optimization of lead compounds and development of formulations suitable for oral administration could facilitate their integration into treatment regimens for both human and animal TB, contributing to the global effort to eradicate TB in alignment with the "One Health" framework.

Overall, this study presents β -aminopropioamidoxime derivatives as promising candidates for new antitubercular therapies, with the potential to address the challenges of MDR TB and zTB infections effectively.

AUTHORS' CONTRIBUTIONS

LK, UJ, VB, KT, and LC: Conceptualization; LK, AU, UJ, VB, KT, and LC: Methodology and investigations; AU, MM, GD, GB, and AY: Chemical experiment and its description; VB, LC, and AA: Microbiological screening for *M. tuberculosis* and its description; KT and AB: Microbiological screening for *M. bovis* and its description; LK and AY: Visualizations and SwissADME. LK: Drafted and revised the manuscript. All authors have read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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