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T.V. Polyudova[1*], T.A. Akentieva[2], M.V. Antipeva[1, 2], A.L. Esaev[1], L.P. Yunnikova[1]

[1]Institute of Ecology and Genetics of Microorganisms, Perm Federal Research Center, Ural Branch of the Russian Academy of Sciences, Perm, Russia
[2]Perm State Agro-Technological University named after academician D.N. Prianishnikov, Perm, Russia
*e-mail: polyudova@iegm.ru
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Toxicity and antimicrobial activity of tropylated aniline and its derivatives

Abstract. This study investigated the biological activity of tropylated aniline 4-(7-cyclohepta-1,3,5-trienyl) aniline and its derivatives belonging to the group of secondary aromatic amines – *N*-2-hydroxyphenyl-methyl-4[1]-(7-cyclohepta-1,3,5-trienyl)aniline and the group of azomethines – *N*-2-hydroxyphenylmeth-ylene-4[1]-(7-cyclohepta-1,3,5-trienyl)aniline. Tropylated aniline was synthesized by mixing tropyllium tetrafluoroborate and aniline in ethanol. *N*-2-hydroxyphenylmethyl-4[1]-(7-cyclohepta-1,3,5-trienyl)aniline and *N*-2-hydroxyphenylmethylene-4[1]-(7-cyclohepta-1,3,5-trienyl)aniline were obtained via a one-pot multicomponent synthesis. Tropylated aniline and its derivatives showed no toxic effects on Galleria mellonella larvae upon invasive administration of 10 µL solutions at a concentration of 1 mg/mL (10 µg/larva). In vitro experiments demonstrated that Gram-negative bacteria and Gram-positive bacteria with hydrophilic cell walls exhibit low susceptibility to tropylated aniline and are resistant to its derivatives. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the studied compounds were determined. The MBC/MIC ratio for all susceptible bacteria was ≤4, indicating their bactericidal activity. A dependence of bacterial susceptibility to tropylated aniline and its derivatives on the level of bacterial cell hydrophobicity was demonstrated. High susceptibility to the studied compounds was revealed in mycomembrane bacteria of the Mycobacterium genus, whose cell walls contain mycolic acids and exhibit a high level of hydrophobicity. The obtained compounds are of practical relevance as potential anti-tuberculosis agents.
Keywords: antibacterial activity, hydrophobicity, tropylidene cycle, mycomembrane, toxicity, *Galleria mellonella.*

Introduction

The rapid spread of multidrug resistance (MDR) among bacteria and micromycetes motivates researchers to continuously search for and develop new safe antibacterial compounds [1]. Globally, a vast number of promising compounds are actively being synthesized or isolated from natural sources; however, many have yet to gain widespread recognition due to limited knowledge of their bioactive potential. Substances exhibiting high antibacterial activity combined with practical applicability are relatively uncommon. Most antibiotics are developed based on existing and known molecular scaffolds, whose functional activity is enhanced through modification of peripheral groups [2]. Nevertheless, the emergence of resistance in contemporary bacteria necessitates the search for novel compounds founded on fundamentally new chemical structures.

Modern organic synthesis strategies demand the production of complex, highly functionalized structures from readily available and simple starting materials in a minimal number of steps. One such key starting compound is 1,3,5-cycloheptatriene (tropilidene), a precursor to a wide range of derivatives [3]. Furthermore, the 1,3,5-cycloheptatriene moiety itself is a biologically active pharmacophore. Its structure is integral to natural products such as thujic acid and thujaplicins, which exhibit a dual spectrum of action–demonstrating both antifungal and antibacterial activity [4].

The phase transition capabilities of molecules can be leveraged in drug design, including for creating agents capable of overcoming transdermal barriers.

Effective antitumor agents, decovine and decocine, have been developed based on alkaloids containing the tropilidene cycle [5].

Tropylation of aniline yields a compound with significantly reduced phytotoxicity, as demonstrated in tests using wheat seeds. Moreover, seed treatment with tropylated aniline substantially enhanced their germination energy [6]. It has also been shown that the low-toxicity compound 4-(7-cyclohepta-1,3,5-trienyl)aniline inhibits the growth of Staphylococcus bacteria and the micromycete Candida albicans. Antifungal and bacteriostatic activity has also been demonstrated for its ditropylated secondary amine derivative, 4-(7-cyclohepta-1,3,5-triehyl)-N-(1-cyclohepta-2,4,6-trienyl)aniline [7].

Consequently, compounds incorporating the tropilidene cycle hold significant interest as potential antibiotic drugs. The absence of toxicity to humans and animals is corroborated by the long-term clinical use of tropilium-containing drugs such as desipramine, peritol (cyproheptadine). It should be noted that the tropylated azomethine – *N*-2-hydroxyphenylmethylene-4^1-(7-cyclohepta-1,3,5-trienyl)aniline and the tropylated secondary amine – *N*-2-hydroxyphenylmethyl-4^1-(7-cyclohepta-1,3,5-trienyl)aniline are classified as low-toxicity compounds [8].

New insights into the biological activity of tropilidene-containing compounds could significantly broaden the prospects for their practical application.

The aim of this work is to study tropylated aniline and its derivatives, obtained via a simple and safe synthetic method, assessing their toxicity using the *Galleria mellonella* larvae model and evaluating their spectrum of antibacterial activity.

Materials and methods

Objects of Study. Compounds: 4-(7-cyclohepta-1,3,5-trienyl)aniline (Compound 1), *N*-2-hydroxyphenylmethylene-4^1-(7-cyclohepta-1,3,5-trienyl) aniline (Compound 2), *N*-2-hydroxyphenylmethyl-4^1-(7-cyclohepta-1,3,5-trienyl)aniline (Compound 3). Stock solutions of the test compounds (10 mg/mL) were prepared by dissolving weighted samples in a 1:1 mixture of ethanol:dimethyl sulfoxide (DMSO).

Synthesis of Compounds. Compound 1 was synthesized by tropylation of aniline with tropylium tetrafluoroborate in ethanol at room temperature [9]. Compound 2 was obtained via a one-step reaction of salicylaldehyde, aniline, and tropylium tetrafluoroborate at room temperature, using ethanol as the solvent [10]. Compound 3 was prepared in a single step by reacting salicylaldehyde, aniline, sodium tetrahydroborate, and tropylium tetrafluoroborate in ethanol at room temperature [11].

Toxicity assessment. Larvae of G. *mellonella* were used [12]. Larvae were obtained from the Educational and Scientific Center for Honeybee Biology at Perm State Agro-Technological University. For each experiment, 30 healthy larvae weighing 0.2 ± 0.02 g were selected, placed in sterile 90-mm Petri dishes, and immobilized by incubation at 4oC for 3 h. Stock solutions of the compounds in the ethanol : DMSO (1:1) solvent mixture (10 mg/mL), diluted 10-fold with sterile deionized water (1 mg/mL), were injected (10 µL) into the hemocoel of G. *mellonella* larvae through the last left proleg using a 30G syringe (BD Micro-FineTM Plus, USA). The larvae were incubated at 37°C for 72 h without feeding. A group of larvae injected with 10 µl of water served as a control. Larval viability was assessed by gentle probing with a blunt needle; larvae that showed no reaction were considered dead.

Bacterial strains. Nineteen bacterial strains from different collections were used (Table 1). Strains were pre-cultured for 20-24 h in liquid nutrient media at their optimal temperatures (Table 1). Cultures were adjusted to a suspension of 106 CFU/mL in sterile nutrient medium for the antibacterial activity (ABA) test for inoculation into plates (10 µL per well).

Antibacterial Activity (ABA). The stock solution (10 mg/mL) was diluted 10-fold with sterile deionized water for ABA testing. The ABA of the 10-fold diluted ethanol:DMSO solvent mixture was also assessed. ABA was determined using the broth microdilution method in Mueller-Hinton Broth (MHB) or Middlebrook 7H9 medium (Table 1) in 96-well microtiter plates [13]. After bacterial inoculation, plates were incubated at the optimal temperature for each strain for 24 h. Growth visualization was performed by adding 10 µL of a 1% solution of 2,3,5-triphenyltetrazolium chloride to each well and incubating for 30 min. Assessment was based on the red coloration of metabolically active cells. The minimum inhibitory concentration (MIC) of the test compounds was defined as the lowest concentration that prevented visible microbial growth. Minimum bactericidal concentrations (MBC) were determined by removing the contents from wells showing no growth, adding 100 µL of sterile nutrient medium to these wells, and incubating the plates for at least 48 h. Bacteria remaining viable after removal of the antibacterial agent resumed growth. The concentration of the compound solution from which no bacterial growth resumed after removal was recorded as the MBC.

Table 1 – Microorganisms used for testing antibacterial activity and their cultivation conditions.

	Species	Strain	Medium for cultivation	Antibacterial test medium	Opt t°C
1	*Bacillus licheniformis*	VKM B-1711D	LB	MHB	37
2	*Corinebacterium ammoniagenes*	IEGM 1862	TSB	MHB	30
3	*Enterococcus faecalis*	NCIMB 13280	TSB	MHB	37
4	*Escherichia coli*	ATCC 25922	LB	MHB	37
5	*Escherichia coli*	M-17	LB	MHB	37
6	*Listeria innocua*	M-2	BHI	MHB	37
7	*Mycobacterium avium*	GISK 168	Middlbrook 7H9	Middlbrook 7H9	37
8	*Mycolicibacterium smegmatis*	GISK 107	Middlbrook 7H9	Middlbrook 7H9	37
9	*Mycolicibacterium smegmatis*	mc^2 155	Middlbrook 7H9	Middlbrook 7H9	37
10	*Proteus vulgaris*	NCIMB 1475	LB	MHB	37
11	*Pseudomonas fluorescens*	ATCC 948	LB	MHB	37
12	*Rhodococcus erythropolis*	IEGM 10	LB	MHB	30
13	*Rhodococcus equi*	NCIMB 10027	LB	MHB	30
14	*Staphylococcus aureus*	ATCC 25923	LB	MHB	37
15	*Staphylococcus cohnii*	VKM 3165	LB	MHB	37
16	*Staphylococcus epidermidis*	ATCC 12228	LB	MHB	37
17	*Staphylococcus epidermidis*	ATCC 29887	LB	MHB	37
18	*Streptococcus pyogenes*	ATCC 8668	TSB	MHB	37
19	*Streptococcus pyogenes*	NCIMB 8884	TSB	MHB	37

Determination of Hydrophobicity. The hydrophobicity of microbial cells was determined using the Microbial Adhesion To Hydrocarbons (MATH) test with n-hexadecane and bacterial suspensions in 10 mM phosphate buffer (pH 7.2), according to the method described by Nachtigall et al. [14].

Statistical Analysis. All experiments were performed in at least three independent trials. Statistical analysis was conducted using StatSoft Statistica 12 software (applying the Mann-Whitney U test) and MS Excel 2010 (calculating mean values and confidence intervals at α=0.05).

Results and discussion

Compound 1 was prepared in one step as shown in Figure 1. The implementation of this synthesis method for 4-(7-cyclohepta-1,3,5-trienyl)aniline (tropylated aniline) enabled the use of non-explosive tropylium tetrafluoroborate instead of tropylium perchlorate, and ethanol as the solvent instead of tetrahydrofuran. The reaction mixture was maintained at room temperature for 3 h. The proposed synthesis method is safer compared to the method described in [15], as it eliminates the use of explosive tropylium perchlorate and employs the less toxic solvent ethanol [9].

Compound 2 was obtained by reacting salicylaldehyde, aniline, and tropylium tetrafluoroborate in ethanol, maintaining the reaction mixture at room temperature for 1 h. The reaction proceeded according to the scheme presented in Figure 2.

Compound 3 was prepared by reacting salicylaldehyde, aniline, sodium tetrahydroborate, and tropylium tetrafluoroborate in ethanol, maintaining the reaction mixture at room temperature for 2 hours. The reaction proceeded according to the scheme presented in Figure 3.

The obtained compounds are hydrophobic, insoluble in water, but readily soluble in the ethanol:DMSO solvent mixture.

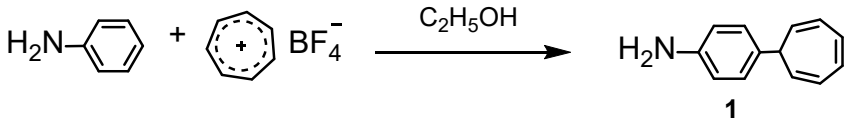

Figure 1 – Scheme for obtaining compound 1 – 4-(7-cyclohepta-1,3,5-trienyl)aniline (tropylated aniline)

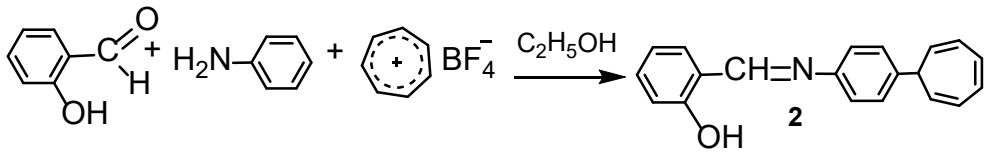

Figure 2 – Scheme for obtaining compound 2 – N-2-hydroxyphenylmethylene-4[1]-(7-cyclohept-1,3,5-trienyl)aniline

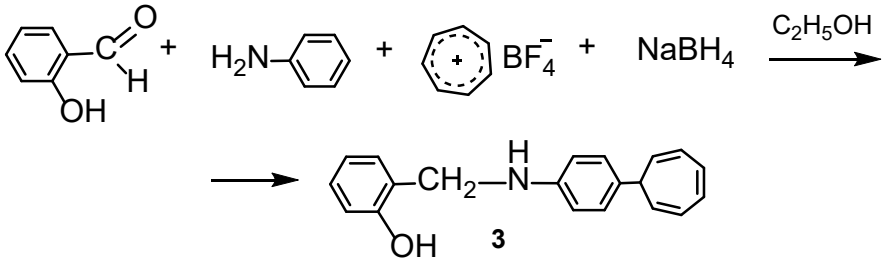

Figure 3 – Scheme for obtaining compound 3 – N-2-hydroxyphenylmethyl-4[1]-(7-cyclohept-1,3,5-trienyl)aniline

Toxicity of tropylated aniline and its derivatives was assessed using *G. mellonella* larvae. *Galleria* larvae provide a rapid and convenient in vivo model for toxicity evaluation, the results of which correlate with mammalian models [12]. The 100% ethanol:DMSO solvent mixture exhibited toxic effects on *G. mellonella* larvae, with only about 40% survival after 3 days. Diluting the solvent mixture 10-fold with water (10%) eliminated any detectable negative effects on the larvae. Tropylated aniline and its derivatives at a concentration of 1 mg/mL in the 10% solvent mixture also caused no toxic effects in the larvae for 3 days following injection into the hemocoel (10 μg/larvae) (Figure 4).

Investigation of the ABA of tropylated aniline and its derivatives revealed inhibitory effects against a broad spectrum of bacteria. Bactericidal activity was also observed for the compounds (Table 2). The Gram-negative bacteria investigated (*E. coli*, *P. fluorescens*, and *P. vulgaris*) exhibited moderate susceptibility (MIC ≥62.5 μg/ml) only to Compound 1 – 4-(cyclohepta-1,3,5-trien-7-yl)aniline, and were resistant to its modified forms. Bactericidal activity of Compound 1 was detected only against *E. coli*, with an MBC 2-4-fold higher than the MIC. The outer membrane of Gram-negative bacteria constitutes a unique protective barrier, incorporating efflux systems for foreign substances. The O-antigen, a polysaccharide with hydrophilic properties located on the outer face of the Gram-negative bacterial membrane, impedes the penetration of hydrophobic compounds through the outer layer [16], which may explain the resistance to tropylated aniline and its derivatives.

As evident from Table 2, the test compounds were primarily active against Gram-positive bacteria. The highest MIC and MBC values were observed for bacteria of the phylum Bacillota. Representatives of the phylum Actinomycetota exhibited greater susceptibility to the test compounds, with the exception of *C. ammoniagenes* IEGM 862. According to [17], an MBC/MIC ratio ≤4 indicates bactericidal activity, while a ratio ≥8 indicates bacteriostatic activity. In cases where MIC and MBC values were precisely determined, the corresponding MBC/MIC ratios were ≤4, further confirming bactericidal action. Only for Compound 2 against *M. smegmatis* GISK 107 was the MBC/MIC ratio equal to 8.

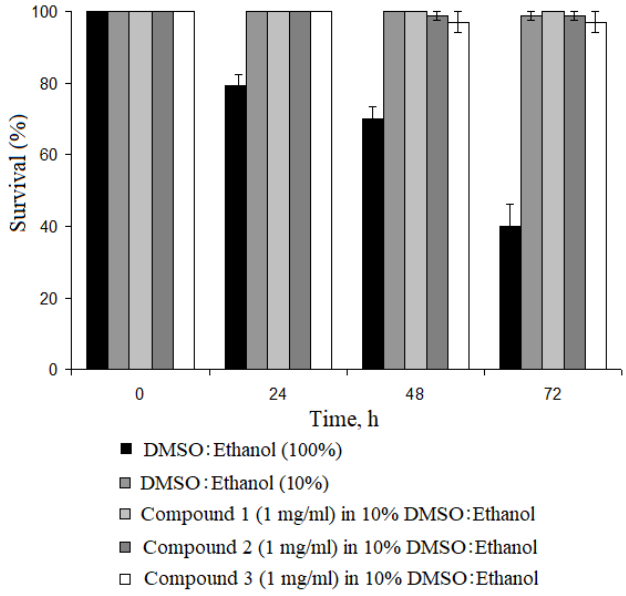

Figure 4 – Survival of *G. mellonella* caterpillars after the introduction of solutions of tropylated aniline and its derivatives into the hemocoel

Table 2 – Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of tropylated aniline and its derivatives, μg/ml

Microorganisms			Compound 1		Compound 2		Compound 3	
			MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria	*E. coli* ATCC 25922		62.5	250	250	>500	>500	>500
	E. coli M-17		125	250	>500	>500	>500	>500
	P. fluorescens ATCC 948		250	>500	>500	>500	>500	>500
	P. vulgaris NCIMB 1475		250	>500	>500	>500	>500	>500
Gram-positive bacteria	Phylum Bacillota	*B. licheniformis* VKM B-1711D	250	500	250	250	62.5	62.5
		E. faecalis NCIMB 13280	250	500	500	500	500	500
		L. innocua M-2	250	250	>500	>500	125	250
		S. aureus ATCC 25923	500	500	250	500	125	500
		S. cohnii VKM 3165	125	250	125	125	62.5	125
		S. epidermidis ATCC 12228	>500	>500	500	500	125	125
		S. epidermidis ATCC 29887	62.5	250	250	500	125	205
		S. pyogenes ATCC 8668	62.5	62.5	500	500	500	500
		S. pyogenes NCIMB 8884	500	500	500	500	250	500
	Phylum Actino-mycetota	*C. ammoniagenes* IEGM 862	125	250	>500	>500	125	125
		R. erythropolis IEGM 10	7.8	7.8	62.5	62.5	3.9	7.8
		R. equi NCIMB 10027	7.8	15.6	31.2	62.5	15.6	15.6
		M. smegmatis GISK 107	1.95	7.8	1.95	15.6	15.6	31.2
		M. smegmatis mc^2 155	1.95	7.8	3.9	15.6	15.6	31.2
		M. avium GISK 168	1.95	7.8	3.9	15.5	15.6	31.2

The efficacy of antimicrobial compounds, their binding to the cell, and penetration intensity depend directly on cell surface characteristics, including hydrophobicity. Assessment of test bacteria hydrophobicity via their adhesion to *n*-hexadecane showed that populations of Gram-negative bacteria lacked a cell fraction exhibiting affinity for this solvent. Minimal hydrophobicity was detected in the Gram-positive bacteria *L. innocua* M-2 (2.7%) and two strains of *S. pyogenes* ATCC 8668 and NCIMB 8884 (3.9% and 2.5%). A low level of hydrophobicity (23%) was found in the *E. faecalis* NCIMB 13280 culture (Table 3). Growth inhibition of Gram-positive bacteria with low cell surface hydrophobicity by tropylated aniline and its derivatives was only observed at high concentrations (250-500 μg/ml).

Table 3 – Hydrophobicity of bacteria according to the MATH test with n-hexadecane

Bacteria		Hydrophobicity, %
Gram-negative bacteria	*E. coli* ATCC 25922	0
	E. coli M-17	0
	P. fluorescens ATCC 948	0
	P. vulgaris NCIMB 1475	0
Gram-positive bacteria	*B. licheniformis* VKM B-1711D	44.0±2.97
	C. ammoniagenes IEGM 862	48.5±7.0
	E. faecalis NCIMB 13280	23.2±2.68
	L. innocua M-2	2.7±1.2
	M. smegmatis mc^2 155	84.8±6.34
	M. smegmatis GISK 107	92.5±2.54
	M. avium GISK 168	90.2±3.32
	R. erythropolis IEGM 10	55.8±2.84
	R. equi NCIMB 10027	88.5±11.3
	S. aureus ATCC 25923	42.0±8.6
	S. cohnii VKM 3165	65.5±11.1
	S. epidermidis ATCC 12228	75.8±9.5
	S. epidermidis ATCC 29887	73.6±10.3
	S. pyogenes ATCC 8668	3.9±1.1
	S. pyogenes NCIMB 8884	2.5±0.8

To evaluate the influence of cell hydrophobicity level on susceptibility to the test substances, the investigated bacteria were divided into two groups based on cell wall type: Gram-positive (Group 1) and Gram-negative (Group 2). Comparison of these groups revealed a significant difference ($p = 0.003$) in the distribution of cell wall hydrophobicity, confirmed by the Mann-Whitney U test (Fig. 5A). Subsequently, the Gram-positive bacteria group (Group 1) was subdivided into two subgroups: one containing only representatives of the phylum Bacillota (Group 1a), and the other containing representatives of the phylum Actinomycetota (Group 1b). Significant differences ($p = 0.012$) in the distribution of cell wall hydrophobicity were also demonstrated between these subgroups of Gram-positive bacteria (Fig. 5B), with Actinomycetota bacteria exhibiting higher cell hydrophobicity levels.

The observed differences in hydrophobicity distribution are attributed to structural features of the cell walls in the compared bacterial groups. It is known that the low hydrophobicity of Gram-negative bacteria is provided by the lipopolysaccharide of the outer membrane. The hydrophobic properties of Gram-positive bacterial cell walls depend on the structure of the peptidoglycan layer. Specifically, the Actinobacteria assigned to Group 1b (*C. ammoniagenes*, *Mycobacterium* spp., *Rhodococcus* spp.) contain mycolic acids in their cell walls. The number of carbon atoms in mycolic acids varies significantly among different bacterial genera. For instance, corynebacteria contain mycolic acids with chain lengths of 20-36 carbon atoms. Bacteria of the genus *Rhodococcus* possess chains of 35-50 carbon atoms, while mycobacteria have mycolates with chain lengths of 60-98 carbon atoms [18, 19]. The structure of mycolic acids determines the permeability of the bacterial cell wall to various compounds and the overall surface hydrophobicity. The peptidoglycan of *Mycobacterium* is covalently linked to arabinogalactan. The termini of arabinogalactan are esterified with mycolic acids, forming the inner layer of the mycobacterial outer membrane, known as the mycomembrane, which dictates the physical and chemical properties of their cell surface [20].

The minimum inhibitory concentrations (MICs) of the test substances were analyzed similarly: a comparison was made between Gram-positive and Gram-negative bacterial groups (Group 1 vs Group 2) (Figure 6), followed by a comparison between the Gram-positive subgroups – Bacillota and Actinomycetota (Group 1a vs Group 1b) (Figure 7).

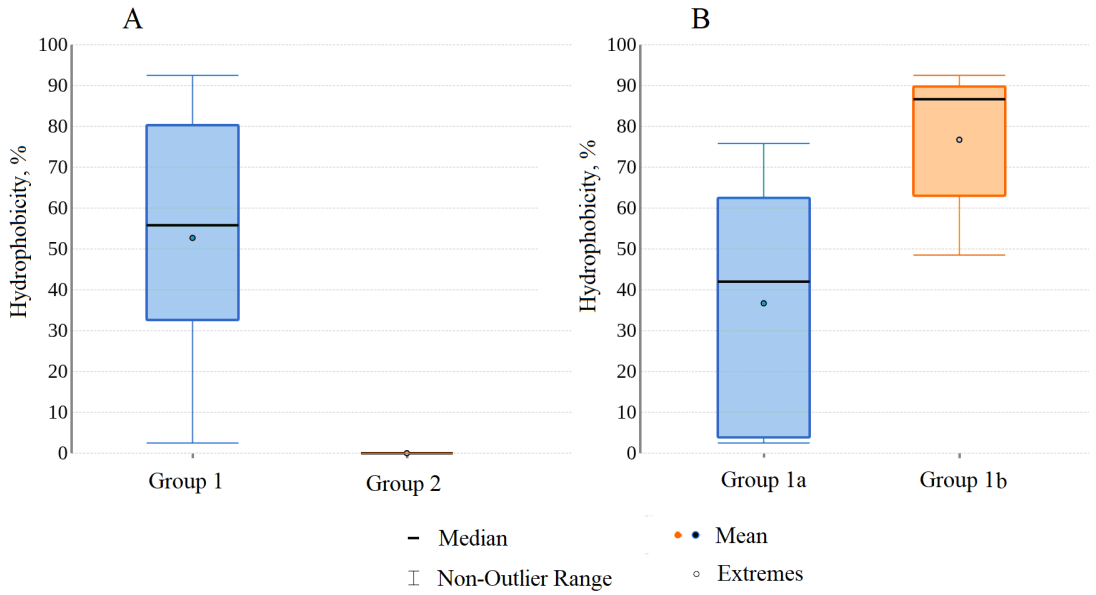

Figure 5 – Distribution of hydrophobicity levels between Gram-positive (Group1) and Gram-negative (Group 2) bacteria (A) and of hydrophobicity levels of Gram-positive bacteria between *Bacillota* (Group 1a) and *Actinomycetota* (Group 1b): Boxplot visualization and Mann-Whitney U-test (A – p=0.003; B – p=0.012)

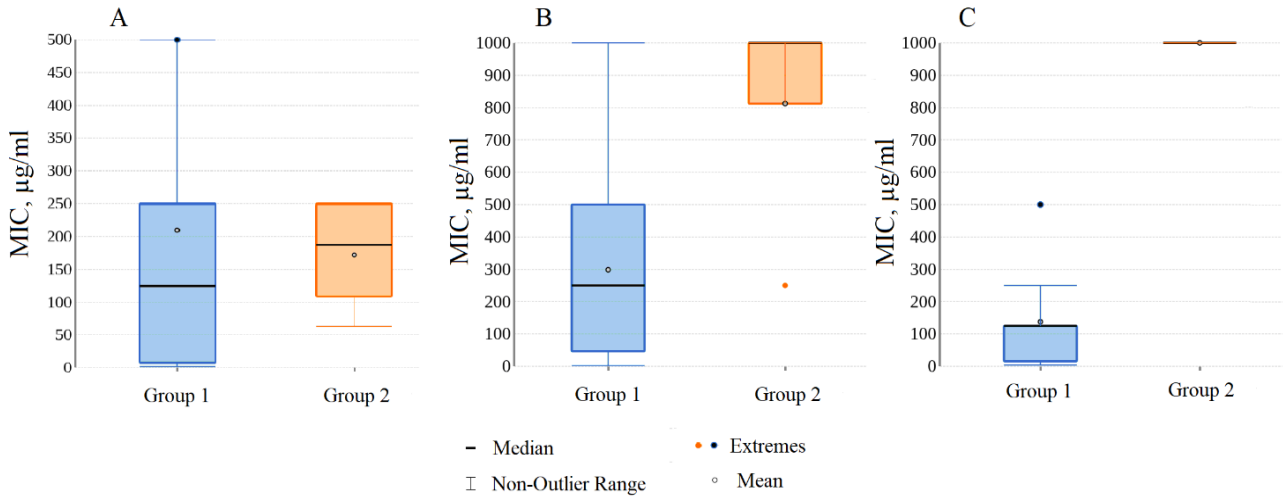

Figure 6 – Analysis of the MICs of Compound 1 (A), Compound 2 (B) and Compound 3 (C) against Gram-positive and Gram-negative bacteria: Boxplot visualization and Mann-Whitney U test. (A – p=0.648; B – p=0,037; C – p=0,003)

When comparing antibacterial effects on Gram-positive and Gram-negative bacteria, analysis of the MICs of the three compounds showed no significant difference in the distribution of MIC values for Compound 1 (p=0.648) (Figure 6A). However, the median MIC values were relatively high, at 125 µg/ml for Gram-positive and 187.5 µg/ml for Gram-negative bacteria. Conversely, the distributions of susceptibility to Compounds 2 and 3 differed significantly between Gram-positive and Gram-negative bacterial groups (p = 0.037 and p = 0.003, respectively) (Figure 6B, C). Gram-negative bacteria exhibited high resistance to both derivatives of tropylated aniline. Gram-positive bacteria, in contrast, were highly susceptible to both compounds, with the greatest susceptibility observed towards the azomethine variant

(Compound 3). Overall, Figure 6 demonstrates that microorganisms with hydrophobic surfaces – the Gram-positive bacterial group – were more susceptible to the test substances than bacteria with hydrophilic cell walls – the Gram-negative microorganism group. This analysis indicates a direct relationship between the level of cell wall hydrophobicity and susceptibility to tropylated aniline and its derivatives.

Comparative analysis of susceptibility within the Gram-positive subgroups (Bacillota vs Actinomycetota) confirmed this observation (Figure 7). The susceptibility distributions for all three compounds differed significantly ($p = 0.004$ for Compound 1, $p = 0.035$ for Compound 2, $p = 0.004$ for Compound 3). Representatives of the phylum Bacillota, with hydrophobicity less than 40%, were more resistant to the test compounds, while the lowest MIC values against all compounds were detected for the most hydrophobic bacteria of the phylum Actinomycetota. Within the Actinomycetota group, the *C. ammoniagenes* IEGM 862 strain possessed the lowest hydrophobicity level and also exhibited the highest, atypical resistance to the investigated compounds. The extremes observed in the box plots of Figure 7 for Group 1b bacteria reflect the MIC values of the test compounds for the *C. ammoniagenes* IEGM 862 strain.

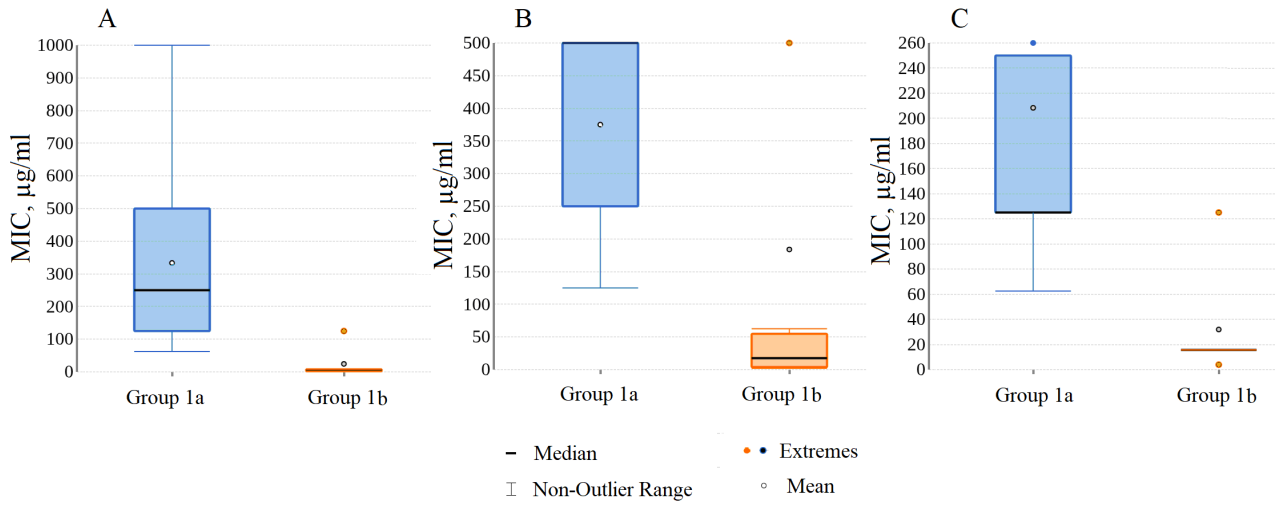

Figure 7 – Analysis of the MICs of Compound 1 (A), Compound 2 (B) and Compound 3 (C) against *Bacillota* (Group 1a) and *Actinomycetota* (Group 1b) Gram-positive bacteria: Boxplot visualization and Mann-Whitney U test (A – p=0.004; B – p=0.035; C – p=0.004)

Thus, the most pronounced susceptibility to tropylated aniline and its derivatives was observed in bacteria with hydrophobic cell walls and high content of long-chain mycolic acids (*Rhodococcus* spp., *M. smegmatis*, *M. avium*), which can constitute 20-50% of the cell dry weight [21].

Tropylated aniline and its derivatives, being structural analogs of *para*-aminobenzoic acid, may competitively inhibit enzymes in the tetrahydrofolate biosynthesis pathway. Tetrahydrofolate is essential for the biosynthesis of purines, thymidylate, methionine, glycine, pantothenic acid, and N-formyl-methionyl tRNA. Inhibition of the folate pathway leads to depletion of tetrahydrofolate, cessation of cell growth, and potential cell death. Compounds with antifolate activity, similar to anticancer drugs (e.g., methotrexate, raltitrexed) and antibacterial agents (e.g., cotrimoxazole), are considered as novel therapeutic approaches for treating infections caused by tuberculous bacteria, including multidrug-resistant strains [22, 23]. The folate biosynthesis pathway offers multiple targets for small molecules with potential for tuberculosis therapy [23, 24].

The tropylium cation, a component of the compounds investigated in this work, may also possess biological activity. The natural compound tropodithietic acid (TDA), known for its broad-spectrum antibacterial action, is relevant. TDA biosynthesis involves dithiol formation, which oxidizes to a disulfide and increases the electron density of the tropylium oxide moiety. Consequently, TDA can chelate metals, providing the chemical basis for its biological

activity. It has been shown that the action of neutral TDA on *E coli* functions via a proton antiporter mechanism. At elevated proton levels, the carboxyl group of TDA picks up H^+, allowing the neutral molecule to diffuse into the cell. In the pH-neutral cytosol, TDA releases the proton. The basicity of TDA, conferred by the tropylium oxide and α-carboxyl group, enables chelation of monovalent cations. This complex diffuses out of the cell, resulting in an exchange of H^+ for a monovalent cation such as K^+ [25].

Conclusion

In conclusion, the non-toxic compounds synthesized via simple reactions of tropylium tetrafluoroborate and aniline demonstrate significant practical potential. The proposed methods for the synthesis of tropylated aniline and its derivatives make it possible to overcome production problems and scalability limitations inherent in other existing approaches. Due to its inherent reactivity, the tropylium cation readily forms covalent bonds with diverse substrates, generating reactive intermediates amenable to further chemical transformations. This positions it as a highly promising precursor for developing novel compounds, including those with broad-spectrum antibacterial activity.

The precise mechanism of antibacterial action for the investigated compounds remains to be fully elucidated. However, the structural features of tropylated aniline and its derivatives suggest potential antifolate activity and/or an ability to disrupt the proton membrane potential, analogous to mechanisms observed in derivatives of *para*-aminobenzoic acid and tropodithietic acid. Critically, the hydrophobicity of the target bacterial surface is a key determinant governing the binding of tropylated aniline and its derivatives to bacterial cells and the subsequent manifestation of antibacterial effects. The high affinity of these compounds for hydrophobic cell walls could form the basis for their use in combination therapies with other agents unable to penetrate such barriers. The potential synergistic effects of tropylated aniline and its derivatives with antibiotics targeting intracellular sites represent an important avenue for future research to identify effective combinations with both novel and established antimicrobial agents. Utilizing chemical compound-antibiotic combinations constitutes a primary strategy for addressing antibiotic resistance. The identified antibacterial effects of the obtained compounds against mycomembrane bacteria indicate their potential as anti-tuberculosis agents.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Information about authors:

Tatyana Polyudova – Candidate of Biological Sciences, Head of the Laboratory of Microbial Development Biochemistry, Institute of Ecology and Genetics of Microorganisms, Perm Federal Research Center, Ural Branch of the Russian Academy of Sciences (Perm, Russia, e-mail:polyudova@iegm.ru)

Tatyana A. Akentieva – Candidate of Chemical Sciences, Associate Professor of the Department of Ecology and Chemical Technologies Perm State Agro-Technological University named after academician D.N. Prianishnikov (Perm, Russia, e-mail: akentieva@hotmail.com)

Marina V. Antipeva – Candidate of Biological Sciences, Junior Researcher, Laboratory of Microbial Development Biochemistry, Institute of Ecology and Genetics of Microorganisms, Perm Federal Research Center, Ural Branch of the Russian Academy of Sciences (Perm, Russia, e-mail: m.antipeva@iegm.ru)

Artem Esaev - Candidate of Biological Sciences, Junior Researcher, Laboratory of Microbial Development Biochemistry, Institute of Ecology and Genetics of Microorganisms, Perm Federal Research Center, Ural Branch of the Russian Academy of Sciences (Perm, Russia, e-mail: a.esaev@iegm.ru)

Lidia P. Yunnikova - Doctor of Chemical Sciences, Professor of the Department of Ecology and Chemical Technology, Perm State Agro-Technological University named after academician D.N. Prianishnikov (Perm, Russia, e-mail: l.yunkova@iegm.ru)

Larisa P. Yunnikova - Junior Researcher, Laboratory of Microbial Development Biochemistry, Institute of Ecology and Genetics of Microorganisms, Perm Federal Research Center, Ural Branch of the Russian Academy of Sciences (Perm, Russia, e-mail: l.yunkova@iegm.ru)