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THIAZOLIDINONE-BASED COMPOUNDS AS DUAL-PURPOSE THERAPEUTICS: ANTIMICROBIAL EFFICACY, CYTOTOXICITY AND PHARMACOKINETIC POTENTIAL

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Infectious diseases and cancer remain leading global health challenges, with rising resistance to existing antibiotics and limited selectivity of many cytotoxic agents. Heterocyclic scaffolds, particularly thiazolidinones, offer a promising platform for the development of novel antimicrobial and anticancer compounds.

The aim of the study. *To evaluate the antimicrobial and cytotoxic properties of thiazolidinone-based compounds against a panel of pathogenic microorganisms and human cancer cell lines, and to identify the most promising derivatives with favorable safety, pharmacokinetic, and mechanistic profiles through molecular docking and dynamics studies.*

Materials and methods. *A library of 5-enamine(hydrazine)-4-thiazolidinone derivatives was screened for antimicrobial activity against Gram-positive and Gram-negative bacteria and Candida albicans, and for cytotoxic activity against six human cancer cell lines. Minimum inhibitory concentrations (MIC) were determined, and IC_{50} values were measured for selected compounds. Pharmacokinetic properties, including gastrointestinal absorption and lipophilicity, were assessed in silico. To investigate potential mechanisms of antibacterial action, molecular docking was performed against MurB (UDP-N-acetylenolpyruvylglucosamine reductase) and DNA gyrase subunit B (ATPase domain), followed by molecular dynamics (MD) simulations to evaluate the stability of the most promising complexes.*

Results*. Thirty-two compounds exhibited antimicrobial activity (MIC ≤ 200 μM), and ten (6, 7, 10, 12, 13, 16, 19, 21, 22, and 29) were identified as the most active. Compound 29, an isatin–oxadiazole hybrid, demonstrated potent activity against Enterococcus faecalis and vancomycin-resistant E. faecium (MIC = 3.13 μM), outperforming vancomycin. Compound 21 was highly active against Staphylococcus epidermidis (MIC = 1.56 μM), while compound 6 showed efficacy against methicillin-susceptible and -resistant S. aureus (MIC = 6.25 μM). Moderate antifungal activity was observed for compound 27 (MIC = 100 μM), whereas Gram-negative bacteria were largely resistant. Cytotoxicity screening revealed selective anticancer activity of compounds 12 and 27, with high therapeutic indices against CCRF-CEM cells and minimal effects on normal fibroblasts. Compound 2 exhibited strong cytotoxicity (IC_{50} = 1.1 μM), while compound 29 combined non-cytotoxicity with favorable pharmacokinetic characteristics.*

Molecular docking supported MurB as the primary antibacterial target, with the most active compounds (21 and 29) showing the most favorable binding energies. Compound 29 also exhibited strong affinity for GyrB, indicating a potential dual-target mechanism. Molecular dynamics confirmed that MurB–compound 29 complexes were particularly stable, correlating well with experimental antibacterial activity.

Conclusions*. Thiazolidinone-based hybrids demonstrated promising antimicrobial and anticancer properties. Compound 29 emerged as a particularly attractive dual-purpose candidate due to its potent activity, safety profile, favorable pharmacokinetics, and validated interaction with essential bacterial enzymes. Together, biological and computational results support the potential of thiazolidinone scaffolds as a basis for the development of selective or multitarget therapeutic agents*

Keywords*: antimicrobial activity, cytotoxicity, pharmacokinetics, molecular docking, molecular dynamics, thiazolidinone, ProTox II*

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1. Introduction

Antimicrobial resistance (AMR) is one of the most pressing global challenges in modern medicine, threatening to undermine decades of progress in combating bacterial infections. The development of next-generation antimicrobials is crucial to addressing infections caused by multiresistant bacteria. Over the next decade, AMR is expected to place significant financial and health burdens on nations worldwide, and the limited supply of medicines accessible will make matters worse [1, 2]. By 2050, multiresistant bacteria are expected to cause more deaths globally than diabetes and cancer combined, emphasizing the urgent need for immediate action to develop and approve new anti-

biotics for clinical use [3]. The World Health Organization (WHO) recommends that one of the most effective short-term strategies for controlling AMR bacteria is the discovery of novel synthetic compounds with antimicrobial activity. Among such promising candidates are 4-thiazolidinone-based derivatives, which have demonstrated affinity for several key bacterial and fungal targets, including beta-lactamases [4], Sortase A (SrtA) [5], peptide deformylase [6], protein mannosyl transferase 1 [7], UDP-galactopyranose mutase (UGM) [7], UDP-N-acetylmuramate/L-alanine ligase (MurC) [8] and dolicholphosphate mannose synthase [9]. It is worthy to mention, 4-thiazolidinone derivatives and their structural analogs have demonstrated significant antimicrobial activity, including efficacy against multiresistant bacteria comparable to standard antibiotics (Fig. 1) [8, 10–16]. Additionally, several thiazole/thiazolidinone-based antimicrobials with promising potential have been identified [17, 18].

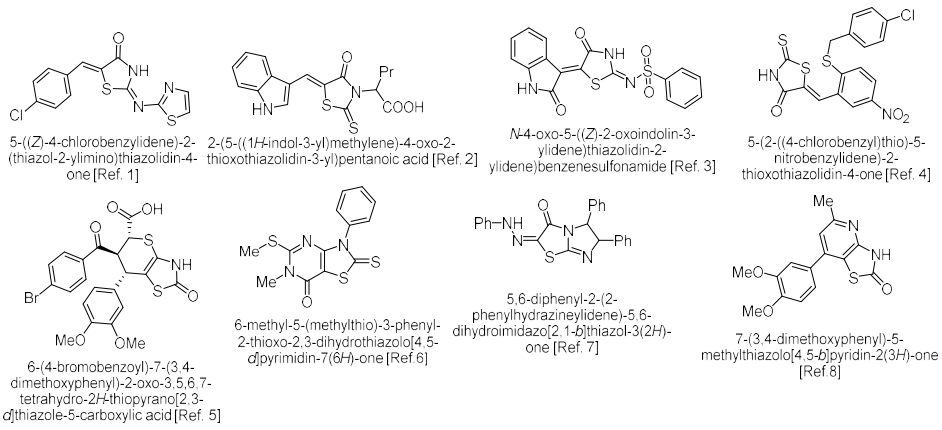


Fig. 1. Structures of selected thiazolidinones and their analogues exhibiting antimicrobial activity

These compounds demonstrate a broad spectrum of biological activity against Gram-positive and Gram-negative bacteria, as well as fungi, owing to the structural versatility of the thiazolidinone scaffold. In this study, 32 compounds representing six distinct series of uncondensed and condensed 4-thiazolidinones and their structural analogues (5-enamine(hydrazine)-4-thiazolidinones, 4-thiazolidinone–pyrazole hybrids, 5-ene-4-thiazolidinones, thiopyrano[2,3-*d*] thiazoles, and isatin-oxadiazole hybrids) were selected for detailed evaluation of their antimicrobial and cytotoxic properties. The selection was based on prior structure–activity relationship (SAR) analyses, which indicated that these derivatives possess favorable pharmacophoric features and improved drug-like characteristics compared to other known thiazolidinone-based compounds. Additionally, previous studies have reported their pronounced antimicrobial, antifungal, antiparasitic, and anticancer potential combined with low cytotoxicity, suggesting that these 32 derivatives represent the most promising candidates for further comprehensive investigation (Fig. 2) [10–16].

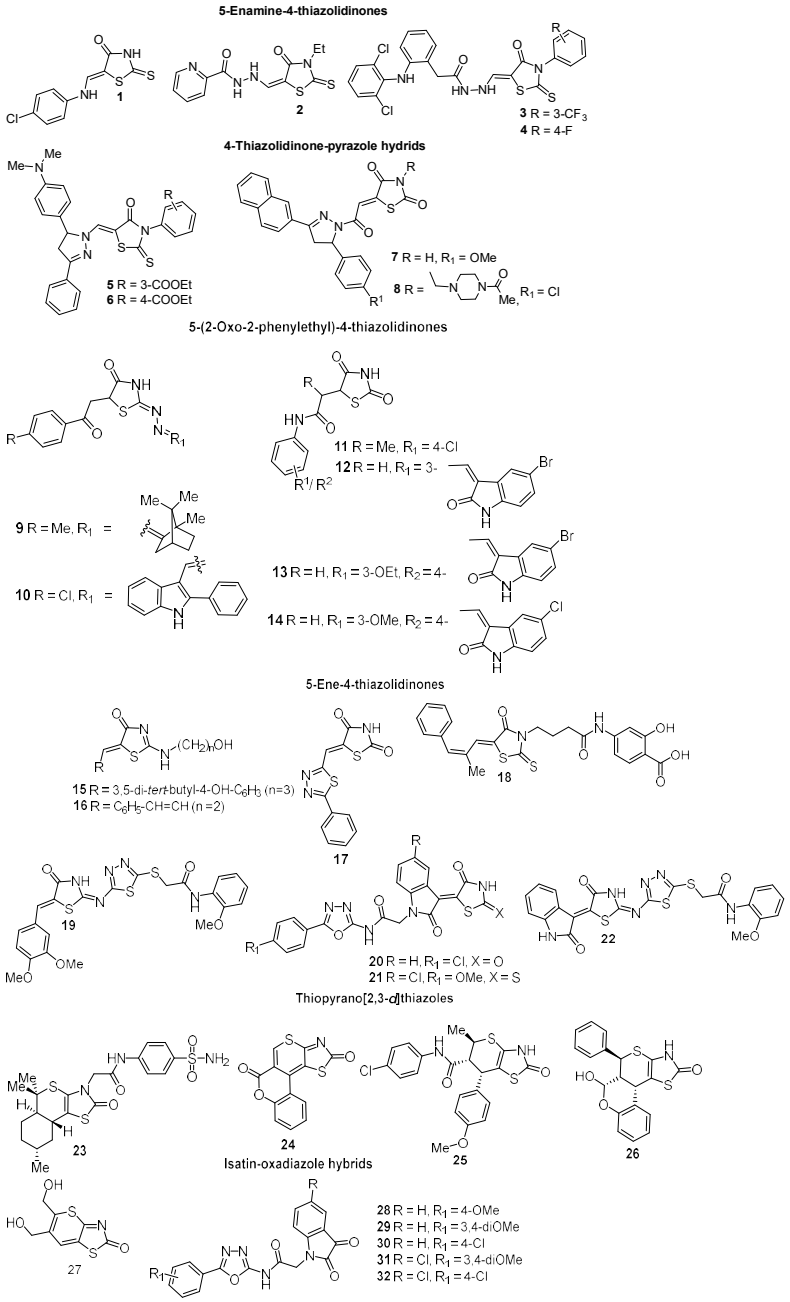


Fig. 2. Structures of 32 derivatives from six different thiazolidinone/thiazole series and their analogues evaluated for antimicrobial activity

2. Planning (methodology) of the research

The study was designed following an integrative approach combining experimental screening and computational modelling to systematically evaluate the antimicrobial and anticancer potential of thiazolidinone-based compounds. The research plan aimed to identify the most promising derivatives with favourable pharmacokinetic, safety, and mechanistic profiles, ensuring reproducibility and predictive analytical value. The methodological framework was structured according to the principles of Quality by Design (QbD), emphasizing systematic planning, outcome orientation, and reliable interpretation of structure–activity relationships.

The planning stage included the following key components: compound selection, *in vitro* activity assessment, pharmacokinetic and safety evaluation, computational modelling, and data integration (Fig. 3). The study focused on a library of 5-enamine(hydrazine)-4-thiazolidinone derivatives, chosen for their heterocyclic scaffolds and potential dual antimicrobial and anticancer activity.

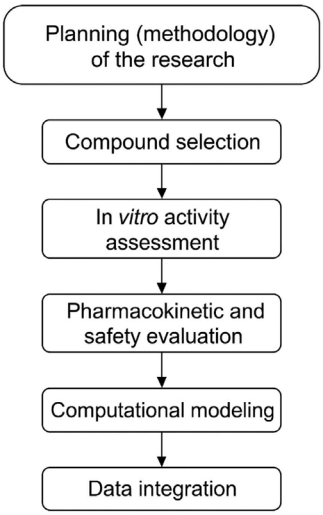


Fig. 3. Experimental design of the study

3. Materials and methods

Test compounds.

The structures and synthesis of the series of thiazolidinone-, thiazole-, and oxadiazole-based compounds were described in our previous studies [19–26]. The experimental studies were conducted over a period of two years.

Pharmacokinetic modeling and toxicity properties.

The physical properties, as well as adsorption, distribution, metabolism, elimination, and toxicity (ADMET) parameters of the compounds, were calculated using the SwissADME online server of the Swiss Institute of Bioinformatics [27]. Organ toxicity (hepatotoxicity) and toxicological endpoints (carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity) were predicted by the ProTox-II software [28].

Cytotoxicity

The cytotoxic effects of compounds were determined using the MTS assay [29–32].

Cell suspensions were prepared and diluted according to the specific cell type to achieve target densities (500–4,000 cells/well). Cells (30 µl/well) were seeded into 384-well clear Corning plates using a MultiDrop Combi dispenser (Thermo Fisher Scientific, USA). After 24 hours, the cells were treated with test compounds, vehicle (DMSO), and high controls (2.67 µM Actinomycin D and 100 µM Mitomycin C) using the ECHO 555 acoustic liquid handler (Labcyte, USA). The compounds were tested at concentrations ranging from 0.012 µM to 50 µM. Following a 72-hour incubation under standard conditions, the MTS solution (Promega) was added to cells in accordance with the manufacturer's instructions. After 1–4 hours, optical density (OD) at 490 nm was measured on a multimode plate reader (EnVision, PerkinElmer, USA). All tests were performed in technical duplicates and in at least three independent biological replicates to ensure reproducibility and statistical validity. The IC_{50} values, the drug concentration lethal to 50% of the treated cells, were calculated from dose-response curves using the Dotmatics software platform. The assay quality was monitored by determining the Z`-factor for each 384-well plate.

Antimicrobial activity.

Eight microorganisms (5 Gram-positive, 2 Gram-negative and one fungal species, *Candida albicans*) were used in the in vitro study. Standard reference bacterial strains (*Enterococcus faecalis* ATCC 29212 = CCM 4224, MSSA *Staphylococcus aureus* ATCC 29213 = CCM 4223, *Escherichia coli* ATCC 25922 = CCM 3954, *Pseudomonas aeruginosa* ATCC 27853 = CCM 3955 and *Candida albicans* ATCC 90028 = CCM 8161) were obtained from the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno.

Additionally, multiresistant bacterial strains were tested, including methicillin-resistant S. aureus (MRSA) 4591/A (PBP2a positive), vancomycin-resistant *Enterococcus faecium* (VRE) VanA phenotype 419/ANA, *Staphylococcus epidermidis* CCM 7221, (a reference strain for biofilm production, ica operon positive), also resistant to fluoroquinolones (DNA gyrase mutation) and resistant to aminoglycosides, resistant to beta-lactam antibiotics and fluoroquinolones and ESBL-positive and resistant to colistin (mcr negative).

Strains without CCM code were obtained from the culture collection of the Department of Microbiology (Faculty of Medicine and Dentistry, Palacký University Olomouc). All tested microorganisms were identified by the MALDI-TOF Biotyper system (Bruker Daltonics, Germany) and stored in cryotubes (ITEST plus, Czech Republic) at –80°C.

The antimicrobial activity of the tested compounds against tested microorganisms was assessed using the standard microdilution method, determining the minimum inhibitory concentration (MIC) as recommended by EUCAST (European Committee on Antimicrobial Susceptibility Testing). Disposable microtitration plates were used for the tests. The compounds were diluted in a MH medium (Mueller-Hinton, BioRad, France) and the plates were inoculated with a standard amount of the tested microbe; the inoculum density in each well was equal to 5×10^5 CFU/mL. The plates were incubated for 18 ± 2 h at 35 ± 1°C, and MICs were determined as the lowest concentration of tested compound that visibly inhibited bacterial

growth [33]. The minimum bactericidal concentration (MBC) is characterized as the minimum concentration of the sample required to achieve irreversible inhibition, i.e., killing the bacterium after a defined period of incubation. To determine MBCs of bacteria and yeast, the contents of the wells with visibly inhibited growth were inoculated onto blood agar and glucose-peptone agar, respectively (Trios, Czech Republic), 1 μL for each well, and incubated for an additional 18 ± 2 h at 35 ± 1°C. Negative growth of microbial colonies within the wells with lowest concentration of tested compounds determined the MBCs.

Molecular docking studies.

Molecular docking studies were conducted to explore the mechanisms of action of the synthesized compounds against MurB (UDP-N-acetylenolpyruvylglucosamine reductase, PDB ID: 1HSK) and DNA gyrase subunit B (GyrB, ATPase domain, PDB ID: 3G7E) [33–49]. 4-Thiazolidinone derivatives are known MurB inhibitors [34–36], while oxadiazole- and isatin-based compounds may target GyrB [37–39]. Docking validation via redocking of FAD (MurB) and B46 (GyrB) yielded RMSD < 2 Å, confirming reliable reproduction of binding poses [40,41]. A known MurB inhibitor ((2*S*)-2-[2-[3-(4-*tert*-butylphenoxy)phenyl]-5-[2-[2-(2-chlorophenyl) ethylamino]-2-oxo-ethyl]-4-oxo-thiazolidin-3-yl]hexanoic acid) [42] and B46 [41] were used as references.

Ligand 3D structures were prepared in Avogadro (MMFF94, 20,000 steps, pH 7.4) [43, 44] and proteins in AutoDock Tools (removal of ligands/water, polar hydrogens, Kollman charges) [45]; FAD was retained in MurB. Structures were converted to PDBQT via OpenBabel [43]. Docking with AutoDock Vina (exhaustiveness = 64) generated up to 10 binding modes within 4 kcal/mol [46, 47]. MurB grids encompassed key residues Ser238, Arg188, Glu308, Tyr187, Lys228, Arg242, His271, Arg225, Tyr149, and Arg310; GyrB grids covered the ATP-binding pocket (Glu50, Asn46, Gly77, Thr165; 55 × 55 × 55 points, 0.375 Å spacing, center x = 37.528, y = 16.222, z = 24.583 Å). Results were visualized in Discovery Studio [47].

Molecular dynamics simulations.

Molecular dynamics simulations were performed in GROMACS [48] via SiBioLead, using AMBERSS9B parameters [49]. Systems were solvated, neutralized (0.15 M NaCl), equilibrated for 1000 ps, and simulated for 100 ns (leap-frog integrator, 5000 frames). Trajectories were analyzed with GROMACS tools and Excel 2019 to assess the stability of selected compounds relative to reference ligands [50].

4. Results

Cytotoxicity.

All compounds were tested *in vitro* for their cytotoxicity against six human cancer cell lines (A549: lung adenocarcinoma, CCRF-CEM: acute T-lymphoblastic leukemia, HCT116 and HCT116p53-/-: colon carcinoma, parental and p53 deficient, K562: chronic myelogenous leukemia, U2OS: osteosarcoma), and two normal fibroblast cell lines (BJ and MRC-5). The results are summarized in Table 1.

The cytotoxic potency of the tested compounds was classified based on their IC_{50} values as follows: high potency (IC_{50} < 10 μM), moderate potency (IC_{50} between 10 and 30 μM), low potency (IC_{50} between 30 and 50 μM), and negligible or no cytotoxicity (IC_{50} > 50 μM).

In the first group of analyzed derivatives of 5-enamine(hydrazine)-4-thiazolidinones (compounds 1 to 4) compound 2 showed very high cytotoxicity against most sensitive cell line, CCRF-CEM (IC50 1.1 μM), as well as against HCT116, HCT116p53-/- and U2OS cells. Selective cytotoxicity against CCRF-CEM was observed for compounds 3 and 4. Compound 1 was inactive, with IC_{50} values exceeding 50 μM for all tested cell lines.

Among the thiazolidinone-pyrazole hybrids 5–8, compounds 5 and 6 exhibited the highest cytotoxic activity, with IC_{50} values lower than those of compounds 7 and 8-specifically, below 5.16 μM against CCRF-CEM and U2OS cells. However, their effects on other cell lines were moderate or weak.

From the 5-(2-oxo-2-phenylethyl)thiazolidinones (9 to 14) compound 12 exhibited high cytotoxic activity against CCRF-CEM, U2OS, HCT116 and HCT116p53-/- cells, and weak cytotoxic effects against K562, A549, BJ and MRC-5 cells. Significant selective cytotoxicity against CCRF-CEM was also observed for compounds 10, 13 and 14 however their effects on other cancer cell lines were weak.

Among the 5-ene-4-thiazolidinones (compounds 15–22), most of the derivatives exhibited weak or negligible cytotoxicity across the tested cancer cell lines. Compound 15 showed low cytotoxicity against all cell lines, with IC_{50} values ranging from 19 to 33 μM. Compound 17 demonstrated selective activity against CCRF-CEM cells (IC_{50} = 12.8 μM) but was inactive against other lines. Compounds 16, 18, and 22 showed no significant cytotoxicity (IC_{50} > 50 μM). Compound 21 displayed weak activity only against CCRF-CEM (IC_{50} = 26.7 μM), while compounds 19 and 20 showed comparable weak activity against the same cell line (IC_{50} = 21.0 μM and 32.2 μM, respectively) but were inactive against other cancer cell lines.

The cytotoxic activities of the thiopyranothiazole derivatives (23-27) were mostly weak. Compound 27 exhibited high cytotoxicity against CCRF-CEM (IC_{50} = 8.41 μM), but weaker activity against HCT116, HCT116p53-/- and U2OS. Compound 23 showed weak cytotoxicity against CCRF-CEM, HCT116 and HCT116p53-/-. Compound 25 induced weak cytotoxicity in all tested cell lines except for non-malignant BJ cells. Compound 26 showed weak cytotoxicity exclusively against CCRF-CEM cells. Among the isatine-oxadiazole hybrids (28 to 32) only compounds 28, 30 and 32 demonstrated weak cytotoxic activity against CCRF-CEM cells. The remaining hybrids were inactive.

Antimicrobial activity.

Among the 41 compounds in our proprietary library, 32 were selected for antimicrobial and antifungal screening based on their structural characteristics and preliminary activity data.

Compounds with an MBC in the range 200–25 μM were classified as having a medium level of activity, while compounds with an MBC < 25 μM were classified as having a high level of activity (Table 2).

Table 1

Cytotoxic activity of the most active compounds (IC_{50}, μM)

Compound	CCRF-CEM	K562	A549	U2OS	HCT116	HCT116p53-/-	BJ	MRC-5	TI (all cell lines)	TI (CCRF-CEM)
1	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.00	1.00
2	1.11	37.37	30.57	11.65	9.7	4.15	> 50	26.89	2.44	34.64
3	12.01	> 50	> 50	> 50	42.05	42.28	> 50	> 50	1.22	4.16
4	13.96	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.14	3.58
5	4.69	37.19	29.45	5.16	29.88	29.6	35.08	24.75	1.32	6.38
6	3.97	34.12	31.83	3.45	31.44	33.39	> 50	30.77	1.75	10.17
7	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.00	1.00
8	32.9	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.06	1.52
9	29.02	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.08	1.72
10	10.48	43.85	28.24	22.77	32.01	31.38	26.35	13.56	0.71	1.90
11	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.00	1.00
12	3.69	25.7	23.3	8	8.27	7.41	40.06	21.78	2.43	8.38
13	10.71	28.28	45.19	23.68	30.88	34.21	> 50	22.84	1.26	3.40
14	11.29	> 50	> 50	36.06	39.11	42.88	> 50	> 50	1.31	4.43
15	19.16	30.39	33.66	27.39	30.26	30.53	27.81	27.18	0.96	1.44
16	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.00	1.00
17	12.85	> 50	> 50	> 50	> 50	> 50	> 50	34.09	0.96	3.27
18	34.11	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.06	1.47
19	21.07	> 50	> 50	> 50	> 50	38.5	> 50	> 50	1.16	2.37
20	32.21	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.06	1.55
21	26.72	> 50	31.57	> 50	28.99	30.5	> 50	> 50	1.38	1.87
22	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.00	1.00
23	20.38	> 50	> 50	> 50	34.61	38.32	> 50	> 50	1.23	2.45
24	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.00	1.00
25	27.45	43.6	33.9	25.48	30.17	32.91	> 50	34.83	1.32	1.55
26	36.63	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.05	1.37
27	8.41	> 50	> 50	41.51	39.12	39.3	> 50	> 50	1.31	5.95
28	42.34	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.03	1.18
29	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.00	1.00
30	34.29	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.06	1.46
31	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.00	1.00
32	38.86	> 50	> 50	> 50	> 50	> 50	> 50	> 50	1.04	1.29

Note: TI (Therapeutic Index) is calculated as the ratio of mean of IC_{50} values in normal fibroblasts to mean of IC_{50} values in all cancer cell lines or only CCRF-CEM cells.

Table 2

In vitro antibacterial and antifungal activity of synthesized compounds, μM (MBC – minimal bactericidal concentration, MIC – minimal inhibitory concentration)

Serie	Compound	Gram-positive bacteria										Gram-negative bacteria				Fungi	
		Staphylococcus aureus 4591 MRSA		*Staphylococcus aureus* CCM 4223 MSSA		*Staphylococcus epidermidis* CCM 7221		*Enterococcus faecium* 419/ANA		*Enterococcus faecalis* CCM 4224		*Escherichia coli* CCM 3954		*Pseudomonas aeruginosa* CCM 3955		*Candida albicans* CCM 8161	
		MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
5-Enamine(hydrazine)-4-thiazolidinones	**1**	> 200	> 200	> 200	50	100	100	n/a	n/a	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
	2	200	200	100	100	200	50	n/a	n/a	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
	3	> 200	> 200	50	50	> 200	200	> 200	200	200	50	> 200	> 200	> 200	> 200	> 200	> 200
	4	> 200	> 200	200	50	> 200	200	> 200	> 200	200	100	> 200	> 200	> 200	> 200	> 200	> 200
Thiazolidinone-pyrazole hydrids	**5**	–	–	25	25	100	25	> 200	> 200	> 200	50	> 200	> 200	> 200	> 200	> 200	> 200
	6	6.25	6.25	12.5	6.25	50	12.5	100	100	25	25	> 200	> 200	> 200	> 200	> 200	> 200
	7	200	12.5	> 200	12.5	> 200	> 200	> 200	25	> 200	12.5	> 200	> 200	> 200	> 200	> 200	> 200
	8	> 200	25	200	25	> 200	25	n/a	n/a	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200

Continuation of Table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
5-(2-Oxo-2-phenylethyl) thiazolidinone	**9**	–	–	50	25	> 200	100	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
	10	200	25	25	6.25	> 200	6.25	> 200	> 200	200	50	> 200	> 200	> 200	> 200	> 200	> 200
	11	> 200	> 200	50	50	> 200	200	n/a	n/a	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
	12	> 200	125	100	12.5	> 200	13.3	> 200	50	> 200	50	> 200	> 200	> 200	> 200	> 200	> 200
	13	n/a	n/a	50	25	> 200	12.5	n/a	n/a	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
	14	> 200	25	> 200	50	> 200	25	n/a	n/a	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
5-Ene-4-thiazolidinones	**15**	–	–	> 200	50	200	100	> 200	100	> 200	50	> 200	> 200	> 200	> 200	> 200	> 200
	16	50	50	100	25	50	12.5	> 200	25	> 200	50	> 200	> 200	> 200	> 200	> 200	> 200
	17	> 200	> 200	> 200	50	> 200	> 200	n/a	n/a	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
	18	50	200	25	25	> 200	200	> 200	> 200	50	20	> 200	> 200	> 200	> 200	> 200	> 200
	19	> 200	50	> 200	50	> 200	12.5	> 200	> 200	> 200	100	> 200	> 200	> 200	> 200	> 200	> 200
	20	n/a	n/a	200	100	50	25	200	100	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
	21	n/a	n/a	> 200	25	> 200	1.56	> 200	100	> 200	100	> 200	> 200	> 200	> 200	> 200	> 200
	22	> 200	> 200	50	25	12.5	12.5	n/a	n/a	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Thiopyranothiazoles	**23**	> 200	> 200	100	50	> 200	200	> 200	> 200	> 200	50	> 200	> 200	> 200	> 200	> 200	> 200
	24	n/a	n/a	> 200	50	> 200	25	> 200	50	> 200	100	> 200	> 200	> 200	> 200	> 200	> 200
	25	> 200	> 200	> 200	25	> 200	> 200	n/a	n/a	n/a	> 200	> 200	> 200	> 200	> 200	> 200	> 200
	26	n/a	n/a	> 200	50	> 200	50	> 200	200	> 200	100	> 200	> 200	> 200	> 200	> 200	> 200
	27	n/a	n/a	> 200	200	> 200	25	> 200	> 200	> 200	100	> 200	> 200	> 200	> 200	100	100
Isatine-oxadiazole hybrids	**28**	100	50	25	25	50	50	100	100	25	25	> 200	> 200	> 200	> 200	> 200	> 200
	29	> 200	> 200	100	100	100	100	3.13	3.13	3.13	3.13	> 200	> 200	> 200	> 200	> 200	> 200
	30	50	50	25	25	50	25	50	50	50	50	> 200	> 200	> 200	> 200	> 200	> 200
	31	n/a	n/a	> 200	> 200	n/a	n/a	> 200	100	50	50	> 200	> 200	> 200	> 200	> 200	> 200
	32	n/a	n/a	> 200	> 200	n/a	n/a	100	50	25	25	> 200	> 200	> 200	> 200	> 200	> 200
Ciprofloxacin (mg/l)		–	–	–	–	–	–	–	–	–	–	0.004	0.004	0.25	0.25	–	–
Vancomycin (mg/l)		0.5	0.5	0.5	0.5	1	1	> 32	> 32	4	4					–	–
Fluconazole (mg/l)		–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.125	–
DMSO		> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200

Note: – – not tested; n/a – not active.

5-Enamine(hydrazine)-4-thiazolidinones showed medium activity against methicillin-susceptible *S. aureus* (MIC = 50–100 μM). Compound 3 demonstrated activity against *E. faecalis* (MIC = 50 μM). Additionally, compound 1 exhibited moderate activity against *S. aureus* (MIC = 50 μM).

Among the thiazolidinone-pyrazole hydrids compound 6 displayed the best activity against staphylococci (MIC = 6.25 μM for both methicillin-resistant and methicillin-susceptible *S. aureus*; MIC = 12.5 μM for *S. epidermidis*). Compound 7 showed activity against staphylococci (MIC = 12.5 μM) and *E. faecalis* (MIC = 12.5 μM).

From the 5-(2-oxo-2-phenylethyl)thiazolidinone derivatives (9 to 14), compounds 10 and 12 showed significant activity against methicillin-susceptible *S. aureus* (MIC = 6.25 and 12.5 μM, respectively) and *S. epidermidis* (MIC = 6.25 μM and 13.3 μM respectively). Both also exhibited medium activity against enterococci (MIC = 50 μM). Compound 13 showed high activity against *S. epidermidis* (MIC = 12.5 μM), while compound 14 showed medium activity against methicillin-resistant *S. aureus* (MIC=25 μM).

Among the 5-ene-4-thiazolidinones derivatives (15 to 21), compounds 21, 19 and 16 showed high activity against *S. epidermidis* (MIC = 1.56 μM for compound 21; MIC = 12.5 μM for compounds 16 and 19). Compound 18 showed high level of activity against *E. faecalis* (MIC = 20 μM), and medium activity against *S. aureus* (MBC = 50 μM). Compound 22 showed high activity against *S. epidermidis* (MIC = 12.5 μM) and methicillin-susceptible *S. aureus* (MIC = 25 μM). All 5-ene-4-thiazolidinones showed medium activity against methicillin-susceptible *S aureus* (MIC = 25–100 μM).

From the isatine-oxadiazole hybrids (28 to 32) compound 29 showed high activity against enterococci, including vancomycin-resistant *E. faecium* (MIC = 3.13 μM). Compounds 28 and 30 showed medium activity against all Gram-positive bacteria, including methicillin-resistant *S. aureus* (MIC = 25–50 μM). Compounds 31 and 32 showed medium activity against enterococci (MIC = 25–100 μM).

Thiopyranothiazole derivatives (23 to 27) showed no significant antimicrobial activity, except for compound 27, which showed antifungal activity against *C. albicans* with MIC of 100 μM.

None of the tested compounds showed significant antimicrobial activity against Gram-negative bacteria.

As result, 10 hit compounds (6, 7, 10, 12, 13, 16, 19, 21, 22 and 29) were identified, with compounds 21 and 29 being the most active.

When comparing MIC and MBC values, a compound is considered more bactericidal if its MIC is closer

to the MBC value [18, 51]. Based on this criterion, the least bactericidal compounds include 5-ene-4-thiazolidinones (21, 19), 5-(2-oxo-2-phenylethyl)thiazolidinone derivatives (10, 12, 13), and thiazolidinone-pyrazole hybrids (7). Among these, compound 21 is likely more bacteriostatic, while compound 29 demonstrates potential as more bactericidal.

It is worth noting that, in most cases, the tested compounds exhibited lower antimicrobial and antifungal activity compared to the reference drugs Ciprofloxacin, Vancomycin, and Fluconazole. An exception was compound 29, which showed significantly higher activity than Vancomycin against *Enterococcus faecium* 419/ANA and *Enterococcus faecalis* CCM 4224.

A general analysis of the screening results from antimicrobial studies highlights compounds 21 from the class 5-ene-4-thiazolidinones and 29 from the class isatine-oxadiazole hybrids. The structure-activity relationship analysis underscores the utility of a hybrid-pharmacophore approach, which successfully combines different pharmacologically attractive fragments – oxadiazole, isatin, and thiazolidinone, into a single molecule with enhanced antimicrobial activity. It is worth noting that such hybrid molecules exhibit significantly greater antimicrobial efficacy compared to their individual substructural components. It should be noted that the condensed thiazolidinone derivatives (thiopyrano[2,3-*d*]thiazoles) exhibited a noticeably lower level of activity compared to the non-condensed analogues.

Although the precise mechanism of action of these compounds remains to be fully clarified, it is important to mention that isatin derivatives, are known to intercalate with DNA and potentially inhibit DNA polymerase III, thereby blocking bacterial replication – one of the key reasons for their antimicrobial efficacy [52]. Based on literature data, isatin-based hybrids represent a promising platform for the development of novel antimicrobial agents with broad-spectrum activity, including activity against resistant strains such as MRSA [51].

Molecular pharmacokinetic and toxicity properties.

SwissADME predictions for the two most active compounds, 21 and 29, revealed their compliance with Lipinski's rule of five (Table 3). Compound 29 satisfies all criteria with zero violations, while compound 21 has one violation due to its molecular weight exceeding 500 g/mol. Both compounds demonstrate optimal lipophilicity, with predicted LogP values of 2.80 (compound 21) and 1.59 (compound 29). The predicted TPSA values ($Å^2$) for synthesized compounds 21 and 29 ranged from 123.86 to 184.05, indicating moderate absorption potential, although compounds with TPSA values above 140 $Å^2$ are generally considered less likely to be orally bioavailable.

Regarding gastrointestinal (GI) absorption, compound 21 was predicted to have low absorption, while compound 29 showed high GI absorption. Both compounds exhibit week blood–brain barrier (BBB) permeability and medium skin permeation values, with predicted log Kp values of –7.38 and 7.68 cm/s for 21 and 29 respectively. Compound 29 is predicted to be a P-glycoprotein substrate, unlike compound 21 (Table 3).

Both compounds were assessed for their potential interaction with cytochrome P450 (CYP) enzymes. Neither compound inhibits CYP1A2, CYP2C19, CYP2D6. However, both compounds are inhibitors of CYP2C9 and CYP3A4.

Table 3

Drug-likeness and toxicity predictions of compounds 21 and 29 computed by SwissADME and ProTox II

Compound / Criteria	**21**	**29**
Formula	$C_{22}H_{14}ClN_5O_5S_2$	$C_{20}H_{16}N_4O_6$
Mol. Wt. (g/mol)	527.96	408.36
NRB	6	7
NHA	7	8
MHD	2	1
TPSA ($Å^2$)	184.05	123.86
LogP (cLogP)	2.80	1.59
Lipinski's rule of five violation	1	0
Skin permeation value (log Kp) cm/s	–7.38	–7.68
GI absorption	Low	High
BBB permeability	No	No
Inhibitor interaction		
P-gp substrate	No	Yes
CYP1A2 inhibitor	No	No
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	Yes	Yes
CYP2D6 inhibitor	No	NO
CYP3A4 inhibitor	Yes	Yes
LD_{50} (mg/kg)	1000	1000
Toxicity class	4	4
Hepatotoxicity	Active	Active
Carcinogenicity	Inactive	Inactive
Immunotoxicity	Active	Inactive
Mutagenicity	Inactive	Inactive
Cytotoxicity	Inactive	Inactive

The organ toxicity (hepatotoxicity) and toxicological endpoints (carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity) of compounds 21 and 29 were predicted using ProTox II (Table 3). The results indicate that both compounds are inactive in terms of carcinogenicity, mutagenicity, and cytotoxicity. However, compound 21 exhibits hepatotoxicity and immunotoxicity, while compound 29 is predicted to be hepatotoxic but not immunotoxic. Although ProTox II predictions provide useful initial insights, the potential implications of the observed hepatotoxicity and immunotoxicity require further investigation. These effects may be dose-dependent and could potentially be mitigated through structural optimization of the compounds to reduce off-target toxicity while preserving biological activity.

Molecular docking.

General correlation between docking and bioactivity. Molecular docking of 32 synthesized 4-thiazolidinone derivatives against MurB and GyrB revealed a correlation between binding affinity and antibacterial activity. Highly active compounds (MIC 6.25–12.5 μM; compounds 7, 10, 12, 13, 21, 29) showed lower AutoDock Vina binding energies, while weakly active or inactive

compounds (MIC ≥ 100 μM; 1, 2, 11, 15, 27) displayed higher energies.

MurB as a potential target. Most active compounds formed stable complexes in the MurB active site, suggesting that their antibacterial activity is at least partially associated with MurB inhibition (Table 4). Compound 21 interacts via π-π stacking, edge-on halogen-π, cation-π, and van der Waals contacts with key residues (Phe274, Arg225, Val239, Gly273), and forms hydrogen bonds stabilizing its binding. Compound 29 also shows strong MurB affinity (Fig. 4).

GyrB as a secondary target. Docking to GyrB (ATPase domain) showed weaker correlation with antibacterial activity (Table 5). Compound 29 binds the ATP-binding pocket with favorable interactions (H-bonds with Gly77 and Thr165, π–anion and π–alkyl contacts), consistent with potential ATP-competitive inhibition. Compound 21 shows moderate interactions (Fig. 5).

Table 4

AutoDock Vina docking scores of tested compounds with MurB

Compounds	Binding energy	Compounds	Binding energy	Compounds	Binding energy
1	–6.066	12	–8.474	23	–7.995
2	–6.942	13	–8.073	24	–7.671
3	–8.084	14	–8.035	25	–7.036
4	–8.386	15	–6.82	26	–7.937
5	–7.956	16	–6.529	27	–5.279
6	–7.806	17	–7.101	28	–8.964
7	–9.309	18	–7.056	29	–9.314
8	–9.804	19	–8.247	30	–9.125
9	–8.112	20	–9.774	31	–9.133
10	–9.56	21	–9.224	32	–8.804
11	–6.898	22	–8.623	Reference ligand	–8.956

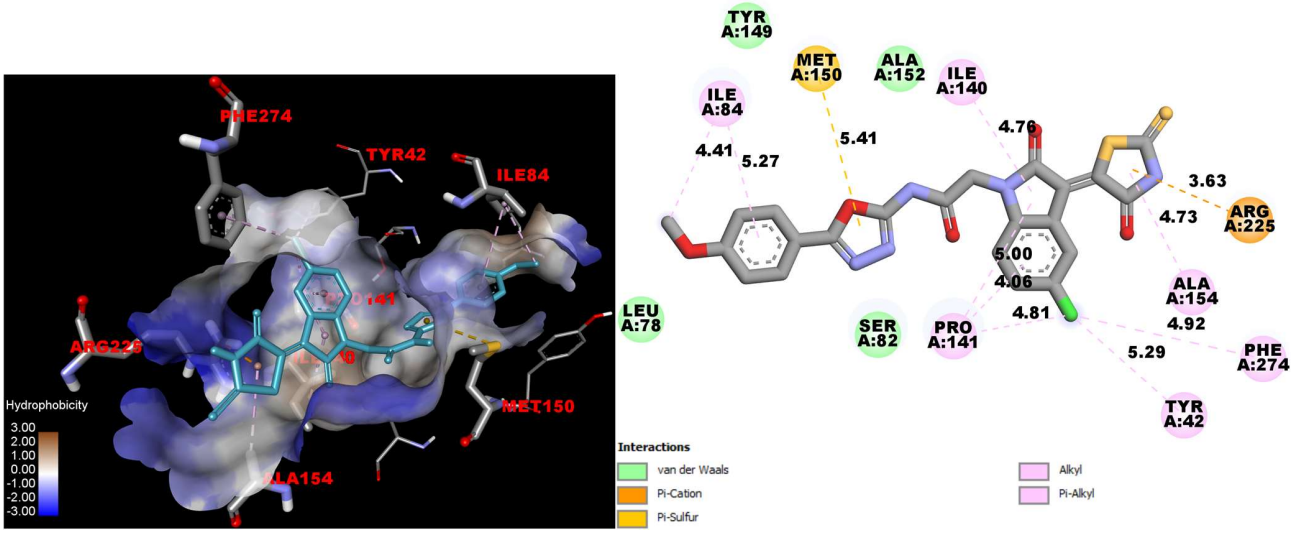
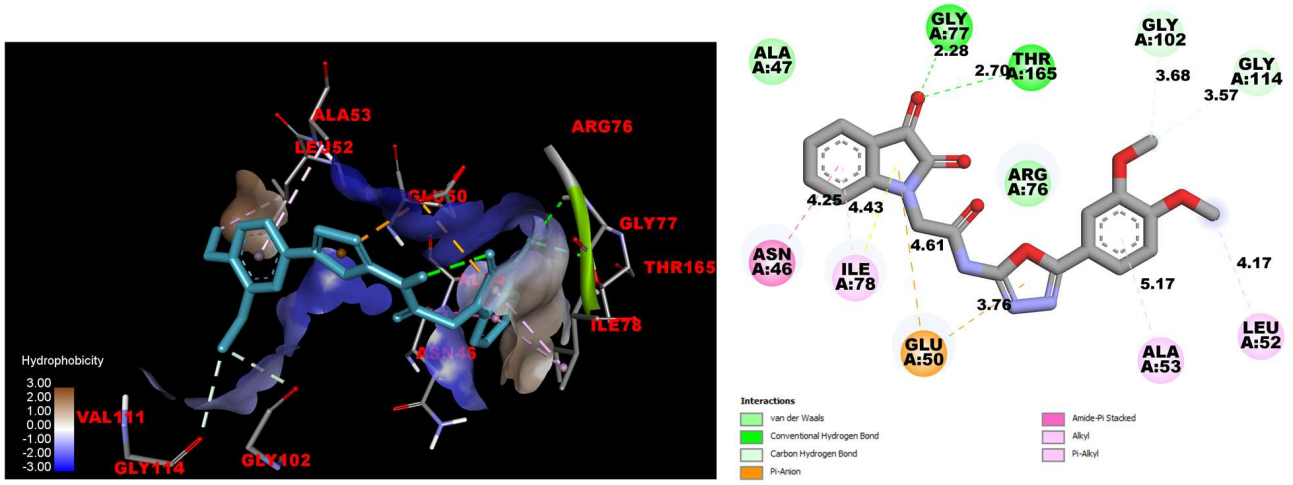


Fig. 4. Predicted binding pose (cyan) and two-dimensional schematic of compound 21 (gray) bound in the active site of MurB (surface colored by hydrophobicity; blue = polar, brown = hydrophobic). Key active-site residues (Arg225, Phe274, etc.) are shown in stick representation



a *b*

Fig. 5. Predicted binding pose of compound 29 within the ATP-binding site of the GyrB subunit: *a* – 3D visualization of the docked complex; *b* – 2D interaction map highlighting hydrogen bonds, π-anion and π-alkyl contacts stabilizing compound 29 in the nucleotide-binding pocket of GyrB

Table 5

AutoDock Vina docking scores for the tested set with the GyrB (PDB 3G7E)

Compounds	Binding energy	Compounds	Binding energy	Compounds	Binding energy
1	–7.105	12	–9.399	23	–8.226
2	–7.704	13	–9.680	24	–8.226
3	–8.847	14	–9.686	25	–9.138
4	–9.585	15	–6.924	26	–8.479
5	–5.677	16	–7.728	27	–6.215
6	–5.499	17	–8.006	28	–9.218
7	–7.193	18	–8.731	29	–10.18
8	–7.052	19	–8.079	30	–10.06
9	–9.145	20	–8.692	31	–9.467
10	–9.071	21	–8.727	32	–9.969
11	–7.876	22	–8.291	**B46**	**–9.976**

Molecular dynamics.

MurB complexes**:** RMSD trajectories equilibrated within 5–10 ns. Compound 29 exhibited the lowest RMSD (~0.10–0.15 nm), indicating stable binding; compound 21 and the reference ligand showed higher RMSD (~0.25–0.35 nm) (Fig. 6). RMSF values indicate minimal fluctuations in active-site residues (<0.2 nm) (Fig. 7). Hydrogen-bond analysis showed compound 29 sustained 2–4 H-bonds, while compound 21 formed fewer and more transient bonds. Radius of gyration remained stable (~2.05–2.15 nm) across all complexes.

GyrB complexes**:** RMSD profiles show B46 as most stable (~0.10–0.15 nm), compound 29 moderately stable (~0.20–0.30 nm), and compound 21 least stable (~0.25–0.35 nm) (Fig. 8). RMSF patterns reveal slightly higher flexibility for compound 21, whereas 29 resembles B46 (Fig. 9). Hydrogen-bond occupancy was lower for compounds 21 and 29 compared to B46. Radius of gyration remained constant (~1.64–1.68 nm), indicating stable global fold.

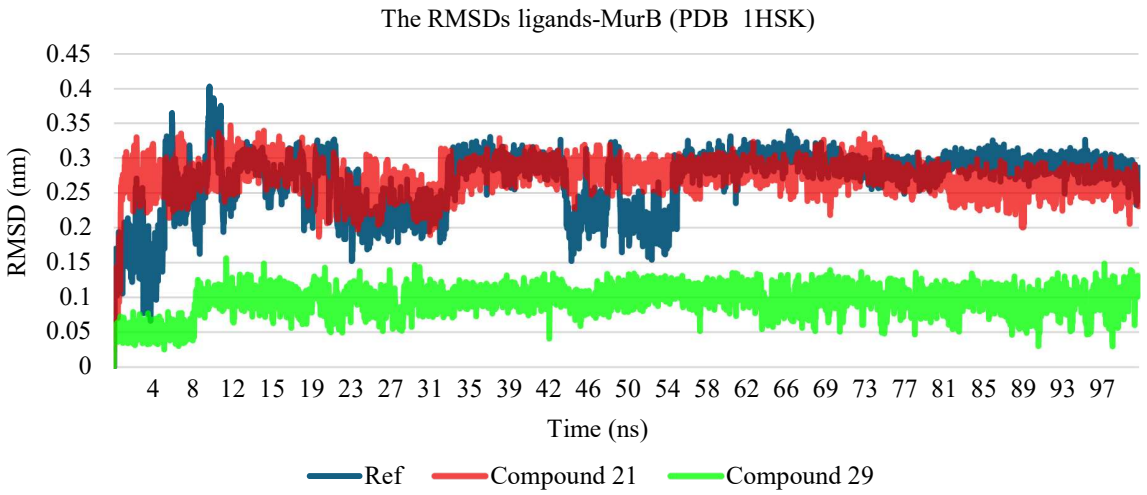


Fig. 6. RMSD dynamics of ligands (reference, compound 21, and compound 29) in complex with MurB (PDB ID: 1HSK) during a 100 ns molecular dynamics simulation

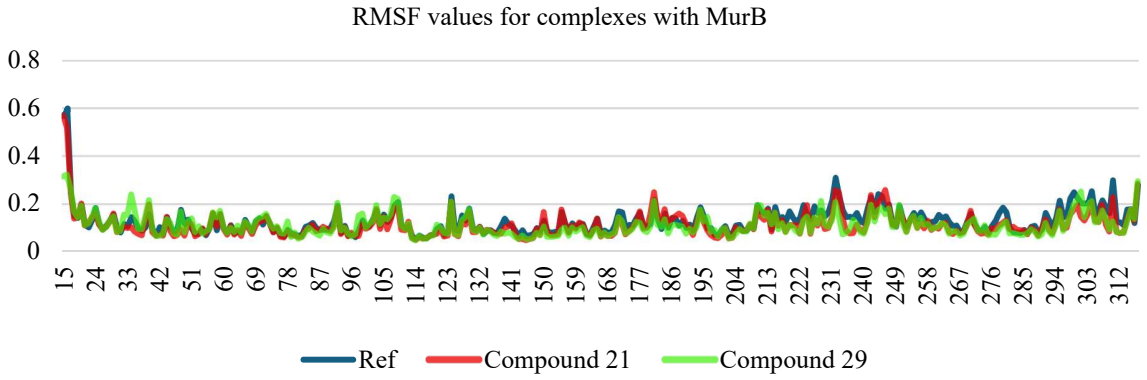


Fig. 7. RMSF values of MurB residues in complexes with the reference literature ligand, compound 21, and compound 29 over the 100 ns molecular dynamics simulation

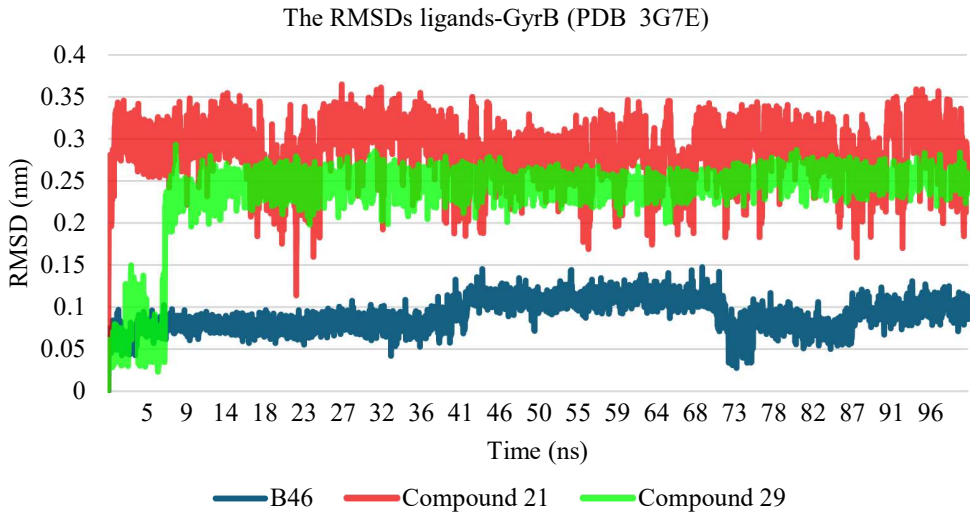


Fig. 8. RMSD dynamics of ligands (B46, compound 21, and compound 29) in complex with GyrB (PDB ID: 3G7E) during a 100 ns molecular dynamics simulation

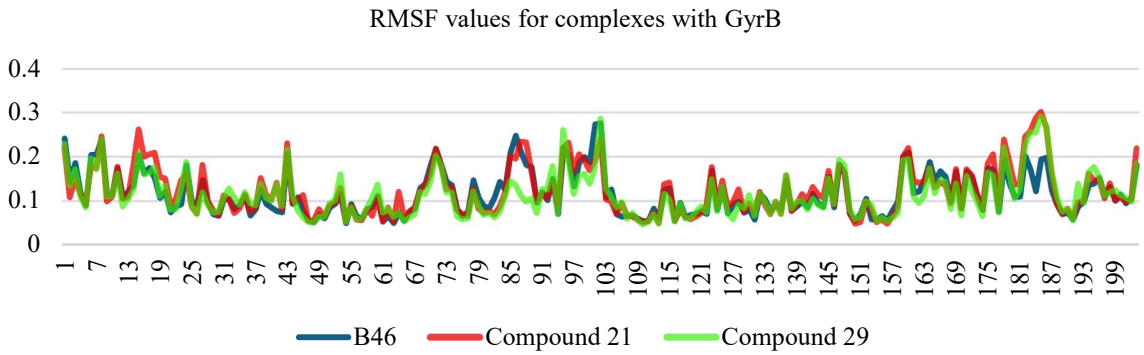


Fig. 9. RMSF values of GyrB (PDB 3G7E) residues in complexes with the B46, compound 21, and compound 29 over the 100 ns molecular dynamics simulation

5. Discussion

Recent investigations have highlighted the therapeutic versatility of thiazolidinone-based derivatives, which have been reported to exhibit both antimicrobial and anticancer properties [10]. Several studies have demonstrated that structural modifications within the thiazolidinone core can significantly enhance antimicrobial efficacy against *Staphylococcus aureus* and *Enterococcus faecium*, while maintaining low cytotoxicity toward mammalian cells [53]. Moreover, molecular docking and dynamics analyses from previous works identified MurB and GyrB as plausible molecular targets responsible for antibacterial activity, consistent with the mechanism proposed in our study [34, 37]. In line with these observations, our results further confirm the dual therapeutic potential of thiazolidinone scaffolds, providing new insights into their structure–activity relationships and pharmacokinetic behavior.

In this section, we provide a detailed assessment of the relationship between cytotoxicity, antimicrobial activity, and pharmacokinetic properties across the tested derivatives. Based on the analysis of cytotoxicity (IC50 values), antimicrobial activity (MIC and MBC values), and pharmacokinetic predictions, three distinct categories of compounds have been identified:

I) Compounds with no cytotoxicity and significant antimicrobial activity. Among evaluated compounds, derivatives 6, 10 and 29 demonstrated high antimicrobial activity (MIC < 6.25 μM) against Gram-positive bacteria, notably *Staphylococcus aureus* and *Enterococcus faecium*. These compounds were non-cytotoxic (IC_{50} > 50 μM) against human cell lines, making them promising candidates for development as selective and safe antimicrobial agents. Pharmacokinetically, compound 29 showed high gastrointestinal absorption and optimal lipophilicity (Log*P* = 1.59), supporting its potential for systemic use.

II) Compounds with cytotoxicity, good selectivity index, and antimicrobial activity. The analysis revealed compound 12 as a noteworthy candidate with selective cytotoxicity against CCRF-CEM cells (IC50 = 3.69 μM) and significant antimicrobial activity (MIC < 12.5 and 13.3 μM) against *S. aureus* and *S. epidermidis,* respectively. Similarly, compound 21 exhibited activity against *S. epidermidis* (MIC = 1.56 μM) while maintaining low cytotoxicity towards normal fibroblasts (IC50 > 50 μM). Pharmacokinetically, compound 21 exhibited moderate absorption, low skin permeability, and no predicted P-glycoprotein substrate activity, enhancing its safety profile.

III) Compounds with cytotoxicity, good selectivity index, and no antimicrobial activity.

For compounds primarily targeting cancer cells, derivatives 2 and 27 displayed high selectivity indices against CCRF-CEM cells, with weak or negligible anti-

microbial activity (MIC > 200 μM). These compounds hold potential for further exploration as anticancer agents without antimicrobial properties. While their pharmacokinetic profiles were not as optimal, further structural modifications could enhance drug-like properties.

Docking results indicate a general correlation between antibacterial activity and MurB binding affinity, supporting MurB as the primary target of the most active compounds (7, 10, 12, 13, 21, 29). The absence of Gram-negative activity likely reflects cell permeability rather than a lack of target engagement.

Compound 21 exhibits favourable π-π and halogen–π interactions with MurB, forming stable hydrogen-bond networks, consistent with its low MIC values. Compound 29 shows strong binding to both MurB and GyrB, supported by hydrogen-bond persistence and low RMSD in molecular dynamics simulations, indicating robust and consistent binding.

GyrB docking results were less predictive, with moderate correlation to antibacterial activity. Some active compounds, including 29, engage the ATP-binding pocket, suggesting possible secondary target effects. MD simulations confirm that compounds 21 and 29 bind GyrB are less stable than the native ligand B46, implying weaker interactions. Overall, MD metrics (RMSD, RMSF, H-bonding, Rg) reinforce that MurB is the main target, with GyrB as a possible secondary target for select compounds.

The combined docking and MD analyses rationalise the observed bioactivity profiles and support the structure–activity relationships inferred from experimental MIC/MBC data.

Practical relevance. Thiazolidinone derivatives, particularly compound 29, show potent antimicrobial activity against Gram-positive bacteria, including vancomycin-resistant strains, and selective anticancer effects. Combining biological assays with molecular docking and dynamics supports mechanism-based design of dual-purpose therapeutics. These findings can guide the development of novel thiazolidinone-based agents with optimized efficacy, safety, and pharmacokinetics.

Research limitations. The study is limited to a specific library of 5-enamine(hydrazine)-4-thiazolidinones and six cancer cell lines. Gram-negative bacteria were largely resistant, and only MurB and GyrB were analyzed as targets, which may not reflect broader activity or alternative mechanisms.

Prospects for further research. Future studies could expand the chemical library, explore additional bacterial and cancer models, and investigate other targets. Fragment-based and dual-target design may further improve antimicrobial and anticancer potency, pharmacokinetics, and safety of thiazolidinone derivatives.

6. Conclusions

Integration of pharmacokinetics with cytotoxicity and antimicrobial analyses underscores the value of hybrid-pharmacophore strategies in drug development. Molecular docking and dynamics indicate MurB as the primary antibacterial target of the synthesized 4-thiazolidinone derivatives, with compounds 21 and 29 showing strong predicted binding affinities that correlate with *in vitro* activity. Compound 29 also binds GyrB, suggesting potential dual-target action, though MurB complexes are more stable, aligning with its activity against fluoroquinolone-resistant *Staphylococcus epidermidis*.

Compounds like 29, with high systemic absorption and low toxicity, exemplify lead structures for optimizing antimicrobial agents, while compounds 12 and 21 highlight dual-purpose potential for targeted therapeutics. Future research should focus on scaffold optimization, mechanism elucidation, in vivo efficacy, and enhancement of therapeutic indices while minimizing off-target effects, demonstrating the role of rational design in developing novel antibacterial and anticancer agents.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this research, whether financial, personal, or authorship-related, that could affect the research and its results presented in this article.

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Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors have used artificial intelligence technologies within acceptable limits to provide their own verified data, which is described in the research methodology section.

Authors' contributions

Dmytro Mural: Investigation; **Andrii Lozynskyi:** Investigation, Writing – Original Draft, Visualization, Methodology; **Victoriya Georgiyants:** Investigation. Olexandra Roman: Investigation; **Anna Kryshchyshyn-Dylevych:** Investigation. Sona Gurska: Investigation; **Pavel Polishchuk:** Methodology, Validation, Formal analysis, Investigation, Visualisation, Writing – Original Draft; **Petr Dzubak:** Methodology, Validation, Formal analysis, Investigation, Visualization, Writing – Original Draft; **Marian Hajduch:** Methodology, Validation, Formal analysis, Investigation, Visualization, Writing – Original Draft; **Katerina Bogdanova:** Investigation; **Kristyna Resova:** Investigation; **Milan Kolar:** Investigation; **Roman Lesyk:** Conceptualization, Methodology, Resources, Validation, Investigation, Writing – Original Draft, Supervision.

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