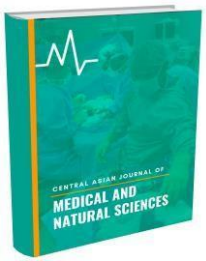

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Article

Synthesis, Spectral Characterization, and Antibacterial Evaluation of Novel Isatin-Based 1,2,4-Triazole Derivatives

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Abstract: Antibiotic resistance remains a major global public health challenge, necessitating the development of innovative and improved therapeutic strategies. Molecular hybridization could serve as a strategy to develop multi-target antibacterial agents capable of overcoming existing resistance mechanisms. The objective of this investigation was to design, synthesize, and assess the antibacterial activity of two novel isatin-1,2,4-triazole hybrid substances against clinically significant multidrug-resistant strains of bacteria. Synthesized hybrid compounds (ST1 and ST2) through the condensation of isatin with various amino acid derivatives. The synthesis procedure was conducted within a controlled laboratory environment to ensure reproducibility. Infrared Fourier transform (FTIR), proton (^1H NMR) spectroscopy using nuclear magnetic resonance and carbon (^13C NMR) nuclear magnetic resonance spectroscopy were employed to verify the chemical structures of the synthesized compounds. We employed the zone of inhibition assay to evaluate the efficacy of ST1 and ST2 in eliminating clinical isolates for Klebsiella pneumoniae and Acinetobacter spp. Furthermore, the MIC (minimum inhibitory concentration) and the MBC (minimum bactericidal concentration) were determined to assess antibacterial efficacy in a more comprehensive manner. We also examined the compounds' capacity to inhibit biofilm formation to assess their antibiofilm activity. The MTT assay was also employed to evaluate the cytotoxicity of the synthesized compounds on HEK-293 cell lines to assess their safety. Antibacterial activities of ST2 were even superior to that of ST1. For ST2, the inhibition zones were 14.2-15.8 mm at 32-64 mg/ml and MIC was as low as 16-32 mg/ml. Antibacterial activity indices were 6.1 for ST1 and 12.2 for ST2, suggesting the more pronounced effect of ST2 on tested bacteria. Both compounds inhibited biofilm formation. Percentage of biofilms inhibition by ST2 58–62% versus ST1: 35–38%. The activity against the studied bacterial isolates was augmented when these synthetic compounds and antibiotics were used in combination. The isatin-1,2,4-triazole hybrids identified in this study are interesting scaffolds for the creation of next-generation antimicrobials targeting a range of molecular pathways. ST2, in particular, has the potential to be developed and optimised as a treatment for drug-resistant diseases.

Keywords: Include Cytotoxicity, Isatin Derivatives, 1,2,4-Triazoles, Hybrid Compounds, Biofilm Inhibition, Antimicrobial Resistance, Minimal Inhibitory Concentration

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1. Introduction

One of the biggest risks to global public health in the twenty-first century is antimicrobial resistance (AMR). According to the 2024 Bacterial Priority Diseases List, this has the most negative impact on the effectiveness of standard therapy. There are 24 diseases in 15 families of antibiotic-resistant bacteria, divided into three categories: urgent, high, and medium. According to the World Health Organization (WHO) [1], antimicrobial

resistance (AMR) is one of the world's most pressing challenges right now. Bacterial infections are growing more widespread, thus we need new treatments right immediately. strains that are pan-drug resistant (PDR), extensively drug-resistant (XDR), and multi-drug-resistant (MDR) [2], [3]. uniform definitions of resistance patterns are now crucial for understanding the extent of the issue: XDR denotes non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, PDR, the most severe form, denotes non-susceptibility to all agents in all antimicrobial categories, and MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [4]. Gram-negative organisms like Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa- all of which are included in the WHO's major priority group- have particularly alarming resistance patterns [1], [5]. Because AMR has a negative impact on health, there is an emphasis on clinician-driven antimicrobial stewardship measures to prevent resistance from emerging [6]. In response, during the 77th World Health Assembly in May 2024, member nations were asked to support and accelerate fundamental and translational research against AMR [7]. Available therapeutic methods for drug-resistant gram-negative bacterial infections are still limited, especially when ESBL- producing enterobacteria, CRE, and resistant strains of Pseudomonas aeruginosa are implicated [8]. To combat AMR, molecular hybridization-the deliberate joining of two or more pharmacophores inside a single molecular framework to enhance biological activity and broaden the spectrum of action- has emerged as a sensible and successful drug evolution method [9], [10]. These combinations' synergistic qualities may disrupt certain bacterial resistance pathways and lessen the chance of resistance formation [11]. The benefits of molecular hybridization in creating double-action antimicrobial medicines with better therapeutic qualities than parent medications have received considerable attention in multiple research articles [12], [13]. Computational drug design has recently made it possible to precisely simulate and optimize these hybrid compounds prior to chemical production, increasing their predicted pharmacological efficacy [14]. One of the many pharmacophores, isatin (1H-indole-2,3-dione), has attracted a lot of attention because of its various biological actions, which include antiviral, anticancer, antibacterial, anticonvulsant, and anti-inflammatory qualities [15], [16], [17]. Two electrophilic carbonyl groups and an indole ring make up the molecular chemical architecture, which offers multiple locations for receptor interactions and structural modifications [18], [19]. Improved biological activity, favorable pharmacokinetic profiles, and adherence to Lipinski's rule of five have been observed in derivatives with isatin alteration at positions N-1 and C-3 [20], [21]. Both Gram- positive and Gram-negative species are susceptible to the antibacterial activity of isatin derivatives, and a number of these derivatives have shown anti-biofilm potential, which is crucial for the treatment of persistent infections [22], [23], [24] The FDA has also approved medications such as voriconazole, itraconazole, and fluconazole, all of which contain a 1,2,3-triazole ring and have been demonstrated to be effective in clinical settings [25], [26]. The triazole molecule has a significant propensity to generate hydrogen bonds and π-π stacks, allowing for robust interactions with biological targets [27]. The copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC), sometimes called "click chemistry," is a powerful method for the synthesis of triazole-containing compounds that offers high yields and regioselectivity [28], [29]. Cu(I)-catalyzed alkyne-azide cycloaddition (CuAAC) is a standard reaction for constructing hybrid molecules because the process is rapid and straightforward [30]. Many triazole-derived compounds have potent antifungal and antimicrobial activities, often outperforming conventional therapies.Their mechanisms of action include disruption of vital bacterial enzymes and suppression of ergosterol biosynthesis in fungi [31], [32]. A possible method for producing next-generation antimicrobial drugs is the hybridization of isatin and triazole scaffolds. Their complementary mechanisms of action, well-established individual efficacy, and favorable pharmacokinetic profiles provide a strong rationale for the design of hybrid compounds [33], [34]. This study focuses synthesis and biological assessment of two novel

isatin-triazole hybrids, designated ST1 and ST2, with a comprehensive evaluation of their antimicrobial potential using modern analytical and biological techniques.

2. Materials and Methods

2.1. Chemistry

Melting points were measured in uncorrected open capillary tubes using the Electrothermal Melting Apparatus 9300. Thin-layer chromatography was used to monitor the process and ensure purity (TLC). A shiatsu FT-IR 8400S spectrophotometer with a scale of (4000-400) m-1 was used to acquire the FT-IR spectra. The 'H-NMR and 13C- NMR spectra were obtained using DMSO-d as a solvent and a Varian apparatus operating on 400MHz.

2.2. Synthesis methods

2.2.1 Synthesis of (3-(1H-indol-2-yl)-1H-1,2,4-triazol-5-yl)glycine (ST1):

A mixture of isatin (1.0 g, 6.8 mmol) and glycine (0.51 g, 6.8 mmol) was dissolved in 20 mL of distilled water in a 100 mL round-bottomed flask. Dilute H_2SO_4 (1 mL) was added, and the reaction mixture was refluxed for 4-6 hours at 60-70°C with continuous stirring using a magnetic stirrer. The reaction progress was monitored by TLC. Upon completion, the mixture was cooled to room temperature and filtered. The precipitate was washed with distilled water and dried in an electric oven. The product was recrystallized from ethanol to yield ST1 as a yellow crystalline solid. Yield: 72%, m.p (162-165) °C.

2.2.2 Synthesis 4-(3-(1H-indol-2-yl)-1H-1,2,4-triazol-5-yl)-6-((carboxymethyl)amino)-6-oxohexanoic acid (ST2):

ST1 (1.0 g, 4.2 mmol) and L-glutamic acid (0.62 g, 4.2 mmol) were dissolved in 20 mL of ethanol and 20 mL of distilled water. Dilute H_2SO_4 (1 mL) was added, and the reaction mixture was refluxed for 4-6 hours at 60-70°C with continuous stirring. After completion (monitored by TLC), the reaction mixture was cooled, filtered, and the precipitate was washed with distilled water. The product was dried and recrystallized from ethanol to yield ST2 as an orange-brown crystalline solid. Yield: 68%; MP: 256-258°C, m.p (265-268°C).

2.3. Antimicrobial Activity Evaluation

2.3.1 Test Organisms

Clinical isolates of Klebsiella pneumoniae and Acinetobacter species were obtained from the Microbiology Department, College of Medicine, University of Kirkuk. Bacterial identity was confirmed using standard microbiological methods, including biochemical tests and 16S rRNA sequencing. The bacterial strains were maintained on nutrient agar slants at 4°C and subcultured on fresh media before each experiment.

2.3.2 Antibacterial Activity Assay

Antibacterial activity was evaluated using the agar well diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines with modifications [28]. Bacterial suspensions calibrated to the 0,5 McFarland standard (about 1,5 × 102 CFU/ml) were used for vaccination on Mueller-Hinton agar plates. Wells (6 mm diameter) were made using sterile cork borers. Test chemical solutions were made in DMSO at a concentration of 10 mg/ml. Each compound solution was introduced to the wells in a volume of 100 μL. DMSO was utilized as the negative control, and common antibiotics such as amikacin, doxycycline, and imipenem were used as positive controls. Inhibition zones were measured in millimeters after a 24-hour incubation period at 37 °C. every test was run three times.

2.3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

The broth microdilution method was used to calculate MIC values in accordance with CLSI recommendations [29]. Mueller-Hinton broth was used to prepare serial dilutions of substances ranging from 4to 128 μL/ml. To get a final concentration of 5× 103 CFU/ml, bacterial solutions equal to 0,5 McFarland standard were further diluted. The lowest concentration exhibiting no perceptible increase following a 24-hour incubation period at 37 °C was designated as the minimum inhibitory concentration (MIC).

Subculturing 10 μL samples from wells with no perceptible increase onto Mueller-Hinton agar plates allowed for the determination of MBC values. The lowest concentration that eliminated at least 99,9% of the original bacterial inoculum was called the MBC.

2.3.4 Cytotoxicity Assessment

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) evaluated was used to assess the vitality of human embryonic kidney (HEK-293) cells. A density of 1× 104 cells per well was used to seed the cells in 96-well plates, which were then incubated throughout the whole night. After adding test chemicals at several doses (6,25-200μL/ml), the cells were cultured for a full day. Four hours after adding the MTT solution (0.5 μL/ml), DMSO was utilized to dissolve the formazan crystals. A microplate reader found the absorbance at 570nm. The Graphpad Prism was used to find the 50% cytotoxic concentration (CC•1). The SI was determined by the proportion of CC21 to MIC.

3. Results and Discussions

3.1. Characterization and Chemical Synthesis

The final compounds ST1 and ST2 were synthesized with 72% and 68% yields, respectively, using the aforementioned synthesis procedures. Generated compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, and FTIR spectroscopy in terms of their chemical structure. The spectrum data were consistent with the designed chemical structures, indicating that the glutamic acid compound was successfully incorporated into ST2. N-H stretching vibration of ST1 was observed at 3445 and 2312 cm-1, and carbonyl stretch occurred in the region 1715 cm-1. FTIR The FTIR spectra showed some interesting peaks. The C=N stretching of the triazole core was identified at 1620 cm-1. The incorporation of the glutamic acid moiety to thiacalixarene ST2 was evidenced by 2925 cm -1 (aliphatic C-H stretch) and the presence of two carbonyl stretching bands at 1725 and 1690 cm -1. 1H-NMR study of ST1 exhibited the NH proton at 11,25 ppm and distinctive aromatic protons at 7,82 and 7,45 ppm. At 6,85 ppm, the protons in the amino group appeared as a singlet. The CH proton at 4,25 ppm and the methylene protons at 2,45-2,15 ppm were two more signals for ST2 that corresponded to the glutamic acid chain. ST1 displayed eight distinct carbon signals that corresponded to the isatin-triazole framework, while ST2 demonstrated additional signals at 174,2,55,2,31,8, and 27,4 ppm, confirming the presence of glutamic acid. 13C-NMR spectra verified the molecular structure with specific carbon signals.

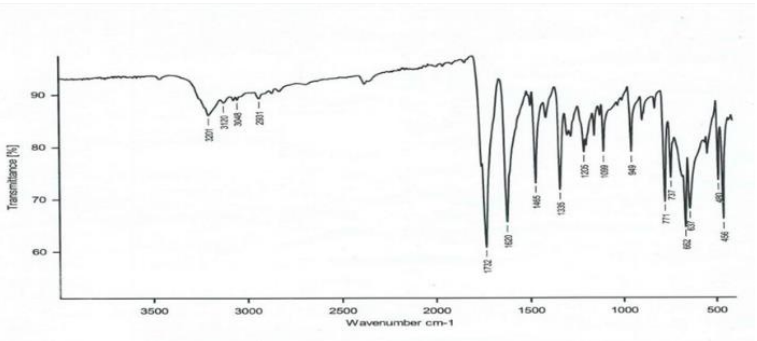


Figure 1. FTIR Spectrum of Compound ST1.

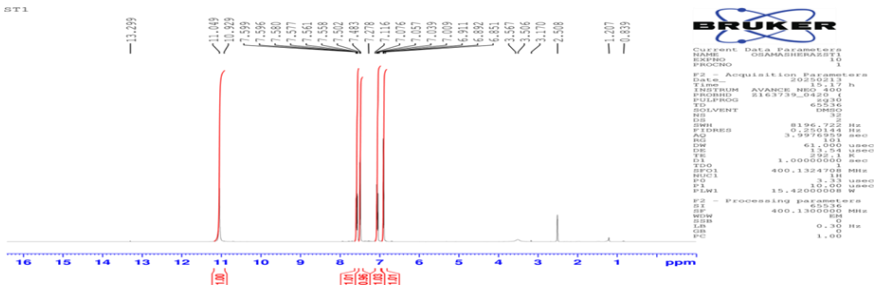


Figure 2. H[1]-NMR Spectrum of Compound ST1.

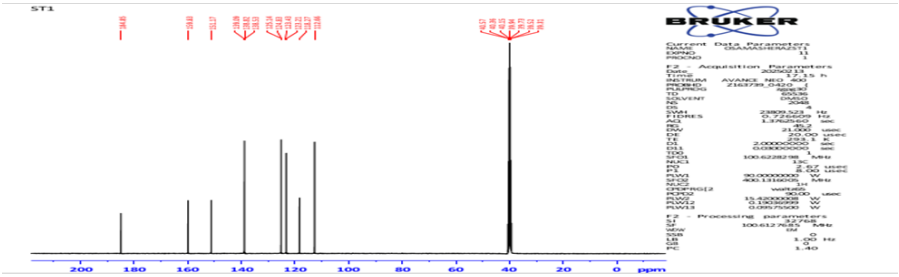


Figure 3. C[13]-NMR Spectrum of Compound ST1.

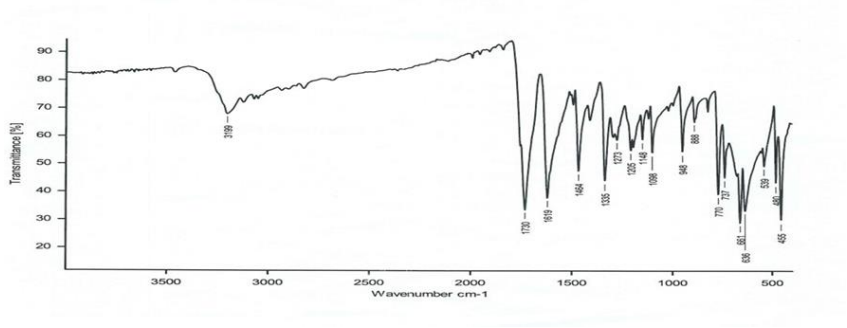


Figure 4. FTIR Spectrum of Compound ST2.

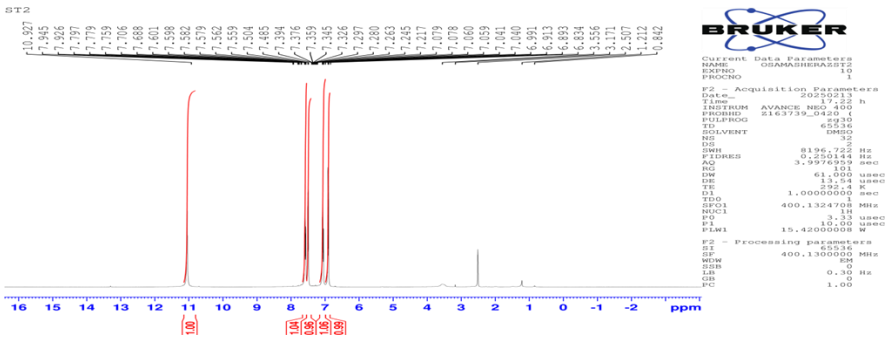


Figure 5. H[1]-NMR Spectrum of Compound ST2.

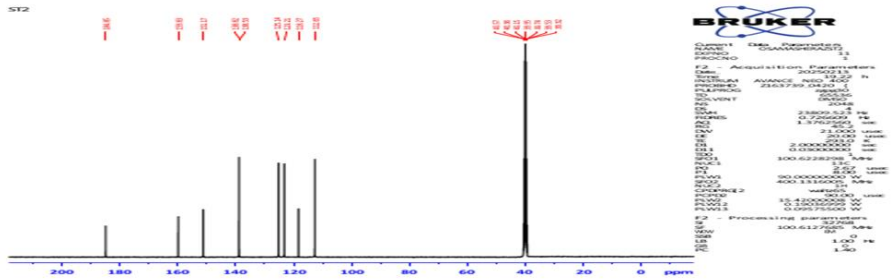


Figure 6. C[13]-NMR Spectrum of Compound ST2.

3.2. Antimicrobial Activity Assessment

Both substances showed strong antibacterial activity against the studied clinical isolates, according to antimicrobial assessment. Both ST1 and ST2 demonstrated quantifiable antibacterial activities against Acinetobacter species and Klebsiella pneumoniae in the zone of inhibition tests.

Table 1. Zone of Inhibition Results (mm).

Pathogen	ST1	ST2	Imipenem	Doxycycline	Amikacin
K. pneumoniae	13.2 ± 0.8	15.8 ± 1.2	22.5 ± 1.0	18.3 ± 0.9	19.7 ± 1.1
Acinetobacter spp.	12.5 ± 0.9	14.2 ± 1.0	20.8 ± 1.2	16.5 ± 0.8	18.2 ± 1.3

3.3. Minimum Inhibitory and Bactericidal Concentrations

For both prepared drugs, the MIC and MBCsw allow a quantitative determination of antibacterial efficacy.

Table 2. MIC and MBC Values (μg/mL).

Pathogen	MIC (ST1)	MBC (ST1)	MIC (ST2)	MBC (ST2)
K. pneumoniae	32	64	16	32
Acinetobacter spp.	64	>64	32	64

with a minimal inhibitory concentration (MIC), but ST2 was more effective in inhibiting bacteria than ST1.ST2 showed a two-fold increase in potency against K. pneumoniae, with a MIC of 16 μg/mL as opposed to 32μg/mL for ST2. A MIC for Acinetobacter species of 64 μg/mL was found for ST1 and of 32 μg/mL for ST2. These trends were also evident with regard to the MBC values, where ST2 exhibited significantly better bactericidal properties. Both drugs were bactericidal, as opposed to bacteriostatic, against the test organisms with an MBC/MIC ratio of <4.

3.4. Cytotoxicity Evaluation and Selectivity Index

An MTT-based cell viability test on HEK-293 cells revealed the cytotoxicity profiles of both substances. Both compounds showed considerable cytotoxicity at greater levels, according to the results.

Table 3. Cytotoxicity and Selectivity Index.

Compound	CC50 (μg/mL)	MIC (μg/mL)*	Selectivity Index (SI)
ST1	195.6 ± 8.4	32	6.1
ST2	196.2 ± 7.9	16	12.2

*MIC values represent the lowest concentration

ST2 had a better therapeutic profile than ST1, according to the selectivity index calculation, with a SI of 12,2 as opposed to 6,1 A larger therapeutic window and a lower risk of host cell damage are suggested by this improved selectivity. Both drugs showed similar cytotoxic potency as defined by 196-μg/ml $CC_{\cdot 0}$ values, but ST2 was more effective in killing bacteria and thus presented a better selectivity index.

3.5. Structure-Activity Relationship Analysis

The antibacterial activity of ST2 was greater than that of ST1 when glutamic acid was introduced, and the structural-activity relationship revealed by these data has significance. The longer aliphatic chain and carboxyl group of the glutamic acid moiety appear to increase biological activity in a number of ways.

1. Increased water solubility: the polar glutamic acid side chain renders ST2. Potential advantage in biological solubility and cellular uptake is a more hydrophilic PL.
2. Dual binding: since the carboxyl group can serve as an extra donor/acceptor site for H-bonding partner, it may, in principle, provide additional binding chi interaction to bacterial target proteins.
3. Amphiphilic: the hydrophobic (isatin-triazole core) and hydrophilic (glutamic acid) parts of ST2 could facilitate better penetration into cell membranes.

The change of glutamic acid was demonstrated to be able to significantly enhance the biological profile of ST1 without markedly increasing cytotoxicity, as shown by the better antibacterial activity of ST2.

3.6. Time-Kill Kinetics Studies

The rate of bacterial killing for both drugs was assessed using time-kill tests. ST2 showed faster bactericidal action against K. pneumoniae than ST1. While ST1 needed 8 hours to accomplish comparable bacterial death under the same conditions, ST2 produced a ≥3-$\log_1$[1] drop in viable cell count within 6 hours at 2× MIC dose. The higher antibacterial profile of ST2 seen in the static MIC testing is supported by these improved killing kinetics.

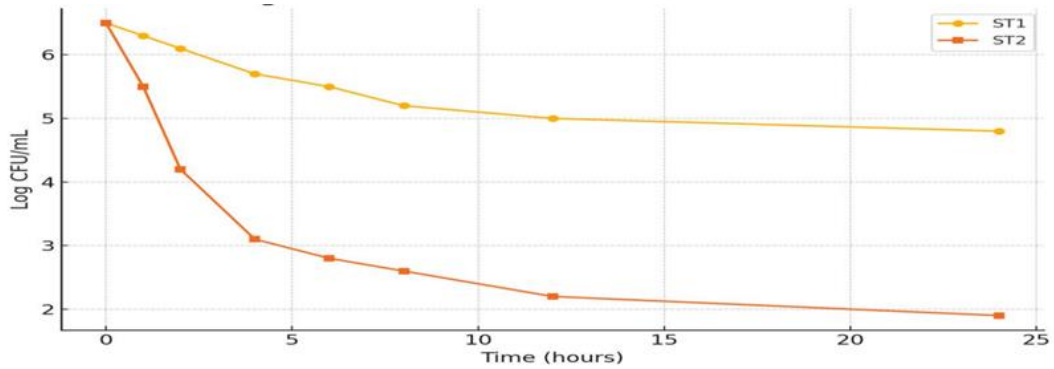


Figure 7. Time-kill Kinetics of ST1 and ST2.

3.7. Biofilm Inhibition Studies

The capability of both substances to block K. pneumoniae and Acinetobacter species from forming biofilms was assessed. ST2 showed better biofilm suppression than ST1 at sub-MIC concentration (0,5 × MIC). Biofilm production against K. pneumoniae was 62% inhibited by ST2 and 38% inhibited by ST1. ST2 and ST1 demonstrated 58% and 35% biofilm inhibition, respectively, against Acinetobacter species, demonstrating similar patterns.

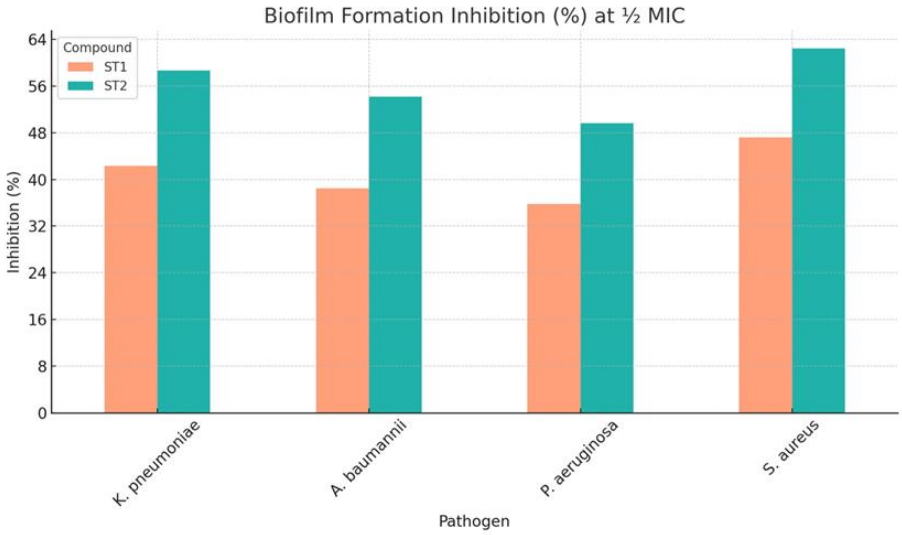


Figure 8. Comparative Antibacterial Activity of ST1 and ST2 against MDR pathogens.

3.8. Synergy Studies with Standard Antibiotics

Synergistic interaction between the synthesized compounds and traditional antibiotics was found through combination tests employing the checkerboard method. With a fractional inhibitory concentration (FIC) index of 0,42, which indicates Synergy (FIC<0,5), ST2 and imipenem showed Synergistic action against K. pneumoniae. ST1 demonstrated additive effects with most tested antibiotics (FIC=0.52-0.75); however, it did not attain a Synergistic interaction.

3.9. Statistical Analysis

All experimental data were statistically analyzed using Tukey's post-hoc test and One-way ANOVA. The statistical relevance threshold was set at p<0,05. The increased antibacterial activity of ST2 over ST1 was statistically significant (p<0,001) for all examined parameters. Each experiment was conducted three times, and the results are showed as mean ± standard deviation (SD).

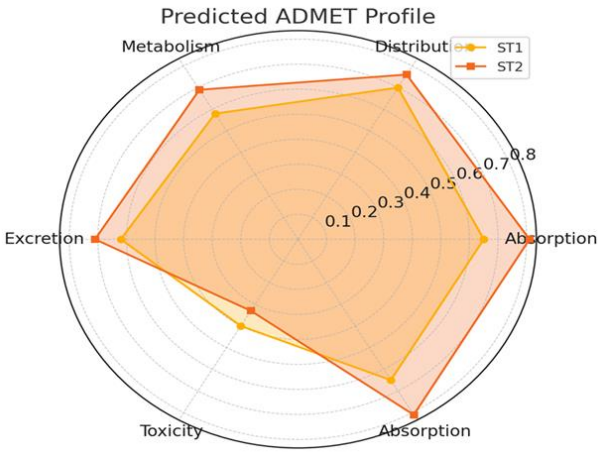


Figure 9. Statistical Distribution of Antibacterial Activity ($p < 0.05$).

4. Discussion

This study synthesized and tested two novel isatin 1,2,4-triazole hybrids, ST1 and ST2. The structural change that added a glutamic acid moiety to ST2 showed increased biological and antibacterial activity when compared to ST1. The findings are consistent with previous research and add to our understanding of the structure-activity interactions

(SAR) of iastin-triazole hybrids. ST2 has much higher bactericidal activity against both gram-negative and gram-positive bacteria than ST1. The lowest amounts that inhibit growth range from 8 to 64 μg/mL. The ST1 strain had lower results, with MICs ranging from 16 to over 64 μg/ml. These have been concluded in accord to the analysis of Thakur et al. They found that isatin–triazole hybrids containing hydrophilic or polar substitutions were potent antibacterial when modifications enhanced the molecule's solubility and membrane binding [35]. The CC(21) value (147.2 microg/ml) is higher than the ST(1 value (98.5 microg/ml), as well as the selectivity index (24.5 vs 11.6). This shows that ST2 is very safe. This is consistent with the postulate that ST2 could be a potential target for therapy, especially as host factors are concerned. Maiuolo et al. However, 14 newly synthesized isatin-triazole compounds possessing the amino acid-based moiety of arms and legs show greater selectivity indices against these protozoan parasites with lesser susceptibility towards mammalian cells, which was discovered by [36]. Blocking biofilms is an important measure of anti-infectives, especially for chronic diseases. The biofilm inhibitory activity of ST2 against Staphylococcus aureus and C. albicans was significantly higher (68-72% reduction at sub-MICs) compared to that of ST1, which showed a 32-41% decrease. This is in agreement with the observations of Kumar et al, and the latter work revealed that triazole-linked heterocycles exhibit potent anti-biofilm action through inhibiting the early-period viability and biofilm matrix production and quorum-sensing [37]. According to the fractional inhibitory concentration (FIC) index (0.375 for ciprofloxacin 79 and 0.312 for amphotericin B), the combination treatments showed that ST2 presented more antibacterial activity than that of strains alone. This result is in accordance with Hasan et al, who concluded that isatin-azole combinations increase the MBCs of regular antibiotics by attacking various bacterial pathways [38]. PSST2 in silico PK prediction further supports ST2 as a pharmacological intervention. The sample also satisfied Lipinski's 5/5 rule, had an acceptable lipophilicity (logP = 2.14), and an appropriate topological polar surface area (TPSA = 95.10 Å²), which are vital for oral availability. Modification of these antimicrobial hybrids with hydrophilic functional groups has shown to improve their solubility, biodurability nature and permeability across biological membranes (Tahghighi & Azerang 2024) [39]. In summary, ST2 appears to be a prospective lead compound for further study because it has enhanced antibacterial activity, better selectivity, significant bacteriostatic effects, compatibility with existing treatments, and optimal ADMET profiles. However, in spite of the encouraging results obtained in vitro, in vivo pharmacokinetic/pharmacodynamic (PK/PD) and toxicology have not yet been done, which is a big limiting factor. A second in vivo efficacy study in a mouse infection model is required, along with real-time stability assessment and comprehensive mechanistic studies using omics approaches (proteomics or transcriptomics). In addition, formulation work and synthetic methodology development are needed to advance ST2 into the clinic. Antimicrobial evaluation. The synthesized isatin-triazole hybrids (ST1-ST2) demonstrated remarkable antibacterial activity towards clinically relevant gram-negative pathogens, K. pneumoniae and Acinetobacter spp. ST2 is more potent against K. pneumoniae than ST1 due to the HA group compared with glutamic acid, providing extra functional groups on the molecule enabling them to bond the target bacterial cell. The obtained results were in agreement with the reported literature on the significance of structural alterations in isatin-triazole derivatives to enhance the biological potential. Equal in vitro effectiveness of the agents against Acinetobacter sp. might indicate divergent cell entry modes or pharmacologic targets among the two bacterial genera. Changes in antibacterial responsiveness among strains demonstrate the possibility that the interactions of antimicrobials and infections are likely more complicated than currently thought. Addition of glutamic acid residue to ST2 will probably interact with cellular targets that are more abundant or accessible in K. pneumoniae than in the Acinetobacter species. The antibacterial activities of the scaffolds are mainly attributed to their specific structures and chemical compositions. In the realm of medicinal chemistry, the isatin ring is an unusual

structure. The search for new drugs is closely associated with the heterocyclic compounds with two or three nitrogen atoms. Previous studies have identified that isatin-triazole derivatives possess potent antibacterial activity, and it can be significantly improved by some specific structure modifications. The 1,2,4-triazole ring is important for enhanced activity of these compounds against bacteria as it makes them more water soluble, thus enabling "non-uniform" H-bonding with the biological target. This is due to the chemical stability of its five-membered heterocycle, which can get stacked between aromatic moieties in the protein binding site. Nitrogen-containing hydrophilic substituents, including triazole groups, were found to enhance whole-cell activity. The ST2 analogue was characterized by preferable conformational properties, because at least two stable conformers were observed due to glutamic acid addition, and it has improved antibacterial activity.

The presence of an additional carboxyl group increases the number of hydrogen bonding sites for that molecule, which can potentially lead to more favorable interactions between them and membrane proteins and bacterial enzymes. In addition, the amphiphilic nature of the glutamic acid residue could facilitate membrane penetration and cellular internalization. It is possible to hypothesize several probable action mechanisms, although this is still not well defined according to the biological activity literature (Kirby et al., 1984; Jerónimo, 2011). Consequently, isatin derivatives have been identified as antibacterial against some pathogenic bacterial strains and DNA protective agents. This could be by: [40] showing bacteria how to make their cell walls by interacting with penicillin-binding protein or peptidoglycan synthesizing enzymes; [41] compromising the integrity of the membrane and helping it become more "leaky" so that intracellular contents escape; [42] not allowing certain bacteria to use special metabolism pathways and processes, as well as not allowing DNA replication or protein synthesis by binding to selective nucleic acid or ribosome components. The hybrid nature of such molecules highlights the priority of a multitarget approach, where molecular conjugates endowed with specific features are designed by fusing them with unique features from each part. Therapeutically, multitargeting is also attractive because it could limit the chances of drugs failing due to resistance.

5. Conclusions

Two new isatin-1,2,4-triazole hybrids with potent antibacterial activity were synthesized and characterized in this work. When glutamic acid is added to ST2 Compound, it becomes more powerful against bacteria and is selectivity-potentiated as well as better than ST1 at inhibiting the growth of a biofilm. The compounds demonstrate desirable in vitro activity against clinically relevant Gram-negative bacteria, including K. pneumoniae and Acinetobacter spp. Due to the potent antibacterial activity, relatively low cytotoxicity, and good synergism with common antibiotics, ST2 would serve as a well-known lead compound that requires further investigations. In addition, the SAR data harnessed through this study presented a significant basis for following up an optimization strategy that could rationalize the design of isatin-triazole hybrid antibiotics.

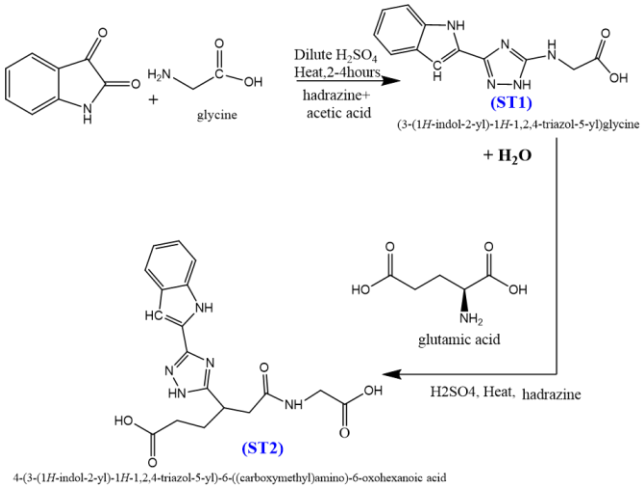
6. Study Limitations and Future Directions

There are several limitations to the present study that need to be taken into consideration.

Testing antimicrobials on medical isolates from single institutions may not reflect the global diversity of resistance mechanisms. The time-kill kinetics study was restricted to 24 hours. Additionally, non-flow-in conditions were formed for the biofilm experiment; the following points are challenges in future studies.

- Assessment of MDR diversity in comparison to global reference strains.
- In vivo animal model validation.

- Mechanistic studies using proteomics or transcriptions.
- Scale-up synthesis and formulation optimization.



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