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Biological Evaluation of Novel Quinazolinone and Spiroquinazolinone Derivatives Synthesized via an Eco-Friendly Surfactant-Mediated Protocol

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Abstract

A library of quinazolinone and spiroquinazolinone derivatives was synthesized through an environmentally benign multicomponent protocol using isatoic anhydride, primary amines, substituted benzaldehydes or isatin derivatives in aqueous medium in the presence of Triton X-100 as a surfactant catalyst. Fifteen compounds, comprising nine quinazolinones (4a–4i) and six spiroquinazolinones (6a–6f), were prepared in good to excellent yields and fully characterized by spectroscopic techniques. The synthesized molecules were evaluated for their antibacterial potential against three clinically relevant bacterial strains: *Escherichia coli* (ATCC 9637), *Pseudomonas aeruginosa* (ATCC BAA-427), and *Staphylococcus aureus* (ATCC 25923). Several derivatives displayed selective antibacterial activity. Compound 4b and compound 4i exhibited the most potent inhibition against *S. aureus* with IC_{50} values of 1.56 µM, while compounds 4i and several spiro analogues (6a, 6b, 6d–6f) demonstrated moderate activity against *E. coli* with IC_{50} values of 12.5 µM. None of the synthesized molecules showed significant activity against *P. aeruginosa* ($IC_{50} > 50$ µM), indicating a marked strain selectivity. Preliminary structure–activity relationship analysis revealed that a para-methoxy substituent and incorporation of tyramine as the amine component were favorable for antibacterial activity.

Keywords: Quinazolines, Antibacterial, Surfactant, Spiroquinazolinones,

1. Introduction

The rapid emergence of multidrug-resistant bacterial pathogens has become one of the most serious threats to global public health. Infectious diseases caused by resistant microorganisms are increasingly difficult to treat, resulting in prolonged illness, greater healthcare costs, and higher mortality rates. Conventional antibiotics such as β-lactams, aminoglycosides, macrolides, and fluoroquinolones have played a central role in modern medicine, but the indiscriminate and prolonged use of these agents has accelerated the development of resistance in both Gram-positive and Gram-negative bacteria. Consequently, the search for structurally novel heterocyclic compounds with improved antibacterial properties remains a major objective in medicinal chemistry. Nitrogen-containing heterocycles occupy a privileged position in drug discovery because of their favorable physicochemical properties and their ability to intearact selectively with a wide range of biological targets. Among these scaffolds, quinazoline and quinazolin-4(3H)-one derivatives have attracted considerable attention owing to their broad spectrum of pharmacological activities. Molecules containing the quinazoline nucleus are known to exhibit antibacterial, antifungal, antitubercular, anticancer, anti-inflammatory, anticonvulsant, antihypertensive, and antiviral properties. Their therapeutic versatility is attributed to the fused benzopyrimidine framework, which provides multiple hydrogen-bonding sites and aromatic surfaces capable of engaging enzymes, receptors, and nucleic acids.

Several marketed drugs contain quinazoline or quinazolinone cores, highlighting the medicinal significance of this heterocyclic motif. For example, Gefitinib and Erlotinib are quinazoline-based anticancer agents used for the treatment of non-small cell lung cancer (Figure 1). Prazosin and Doxazosin are quinazoline

derivatives widely prescribed for hypertension and benign prostatic hyperplasia. These successful examples demonstrate that relatively small structural modifications around the quinazoline nucleus can produce profound changes in biological activity and target selectivity. Quinazolin-4-one derivatives are particularly attractive because the carbonyl group at the 4-position and the ring nitrogens generate a highly functionalized pharmacophore capable of binding to diverse biomolecular targets. Numerous synthetic analogues have been reported to possess potent antibacterial and antifungal activities. Substitution at the 2- and 3-positions of the quinazolinone ring often exerts a strong influence on antimicrobial potency, lipophilicity, and selectivity. Electron-donating and electron-withdrawing groups on appended aromatic rings can further modulate interactions with microbial enzymes and membrane-associated proteins.

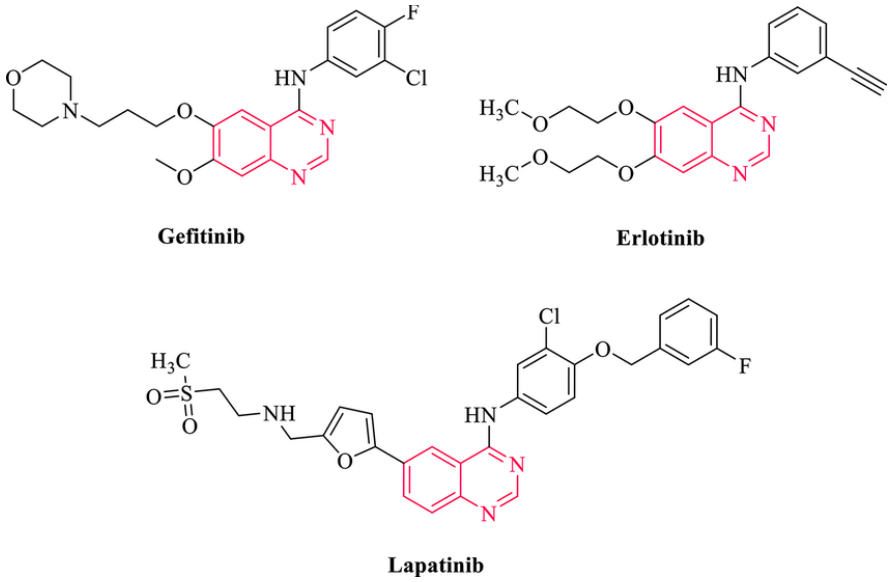

Figure 1: Structures of few quinazolinone based FDA approved marketed drugs

In the present study, a focused library of fifteen compounds was synthesized using a surfactant-assisted one-pot protocol in aqueous medium, consisting of nine quinazolinones (4a–4i) and six spiroquinazolinones (6a–6f) as shown in figure2a and 2b. Structural diversity was introduced through different substituted benzaldehydes, primary amines, and isatin derivatives in order to investigate the influence of electronic and steric effects on antibacterial activity. The synthesized compounds were evaluated against three clinically relevant bacterial strains: *Escherichia coli* (ATCC 9637), *Pseudomonas aeruginosa* (ATCC BAA-427), and *Staphylococcus aureus* (ATCC 25923).

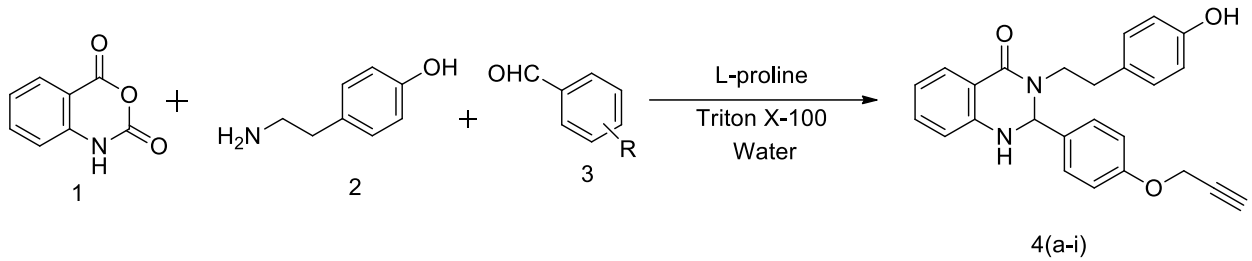

Figure 2a: Synthesis of quinazolinones 4a-i

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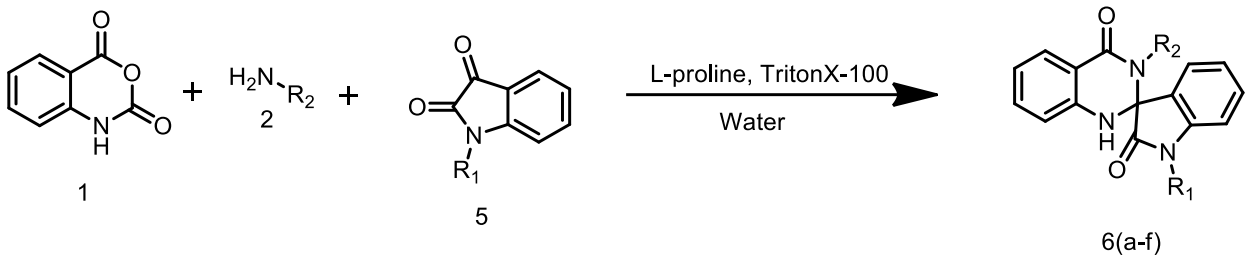


Figure 2b: Synthesis of spiroquinazolinones

The objectives of this work were: (i) to examine the antibacterial potential of newly synthesized quinazolinone and spiroquinazolinone derivatives; (ii) to identify structural features associated with enhanced biological activity; and (iii) to assess the utility of a sustainable synthetic methodology for generating pharmacologically relevant heterocycles. The results demonstrate that selected derivatives possess promising and highly selective antibacterial properties, with inhibitory concentrations as low as 1.56 µM against *S. aureus*. These findings support the continued exploration of quinazolinone-based scaffolds as potential leads for the development of new antimicrobial agents.

2. Materials and Methods

2.1 Chemistry and Test Compounds

The quinazolinone and spiroquinazolinone derivatives evaluated in the present study were synthesized according to the environmentally benign multicomponent procedures developed in the synthetic part of this work. The methodology employed isatoic anhydride as the key heterocyclic precursor, which was reacted with a series of primary amines and either substituted benzaldehydes or isatin derivatives in aqueous medium using Triton X-100 as a nonionic surfactant catalyst. This protocol enabled the efficient one-pot construction of highly substituted quinazolinone and spiroquinazolinone frameworks under mild reaction conditions

A total of fifteen compounds were prepared and selected for biological evaluation. The test set consisted of nine 2,3-disubstituted quinazolin-4(3H)-one derivatives (4a–4i) and six spiro[indoline-3,2′-quinazolin]-2,4-dione derivatives (6a–6f). Structural diversity was introduced by varying the electronic nature of the aromatic aldehydes and by employing different primary amines such as aniline derivatives, cyclohexylethylamine, and tyramine. All compounds were purified by recrystallization or column chromatography and their structures were confirmed by standard spectroscopic techniques including infrared spectroscopy, ^1H NMR, ^13C NMR, and mass spectrometry. The purity of each compound was considered satisfactory for biological testing.

2.2 Microorganisms Used for Antibacterial Screening

The synthesized compounds were evaluated against three well-characterized bacterial strains obtained from the American Type Culture Collection (ATCC). These included:

1. *Escherichia coli* ATCC 9637 (Gram-negative)
2. *Pseudomonas aeruginosa* ATCC BAA-427 (Gram-negative)
3. *Staphylococcus aureus* ATCC 25923 (Gram-positive)

These organisms were selected because they represent clinically important pathogens associated with a wide range of community-acquired and hospital-acquired infections. *P. aeruginosa* is particularly recognized for its intrinsic

resistance to many antibiotics, while *S. aureus* remains one of the most important Gram-positive pathogens in human disease.

2.3 Preparation of Test Solutions

Stock solutions of each synthesized compound were prepared in dimethyl sulfoxide (DMSO) or another suitable sterile solvent to obtain concentrations adequate for serial dilution. The final concentration of solvent in the assay medium was maintained at a level that did not interfere with bacterial growth. Appropriate control experiments containing the solvent alone were performed to confirm the absence of antibacterial effects due to the vehicle.

2.4 Antibacterial Assay

The antibacterial potential of the synthesized compounds was determined by measuring the concentration required to inhibit 50% of bacterial growth (IC_{50}). Bacterial cultures were grown under standard laboratory conditions in nutrient broth or a suitable growth medium until they reached logarithmic phase. The cultures were then diluted to a standardized inoculum density and exposed to varying concentrations of the test compounds.

After incubation for an appropriate period under controlled temperature conditions, bacterial growth was quantified spectrophotometrically or by another standard microbiological method. The percentage inhibition of growth at each concentration was calculated relative to untreated control cultures. IC_{50} values were derived from concentration–response curves and are reported in micromolar (μM) units.

3. Results and Discussion

3.1 Antibacterial Evaluation of Quinazolinone and Spiroquinazolinone Derivatives

A focused library of fifteen heterocyclic compounds comprising nine quinazolinone derivatives (4a–4i) and six spiroquinazolinone analogues (6a–6f) was evaluated for antibacterial activity against three clinically relevant bacterial strains: *Escherichia coli* (ATCC 9637), *Pseudomonas aeruginosa* (ATCC BAA-427), and *Staphylococcus aureus* (ATCC 25923). The antibacterial potency was expressed as IC_{50} values in micromolar units (μM), representing the concentration required to inhibit 50% of bacterial growth. The complete screening data are summarized in Table 1 mentioned below. The biological screening revealed that the synthesized compounds displayed selective and strain-dependent antibacterial activity. Most of the derivatives were inactive at concentrations above 50 μM against at least one of the tested organisms, indicating that subtle structural changes had a pronounced effect on biological performance. Nevertheless, several compounds demonstrated moderate to excellent inhibitory activity against *E. coli* and *S. aureus*, with IC_{50} values as low as 1.56 μM.

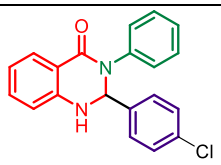
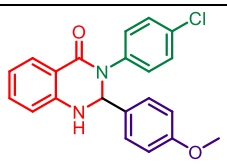
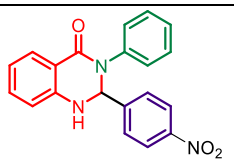
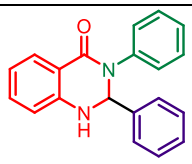
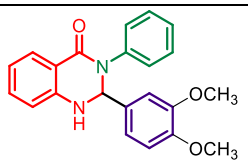
3.2 Activity Against *Escherichia coli* (ATCC 9637)

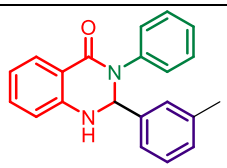
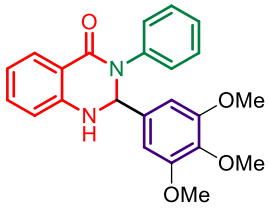
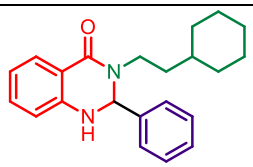
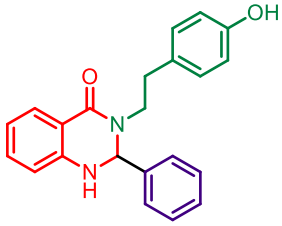
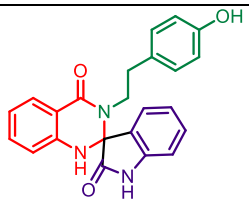
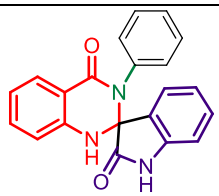
Among the screened compounds, several derivatives exhibited measurable activity against the Gram-negative strain *E. coli*. Compounds 4a and 4b produced moderate inhibition with IC_{50} values of 50 μM. A more pronounced effect was observed for compound 4i, which inhibited bacterial growth with an IC_{50} value of 12.5 μM. This result suggests that incorporation of tyramine as the amine component substantially enhanced antibacterial activity compared with simpler aniline-derived analogues. The spiroquinazolinone series also demonstrated selective activity against *E. coli*. Compounds 6a, 6b, 6d, 6e, and 6f each displayed IC_{50} values of 12.5 μM, indicating moderate antibacterial potential. In contrast, compound 6c and most substituted quinazolinones were inactive ($IC_{50} > 50$ μM). These findings indicate that the spirocyclic framework is compatible with antibacterial activity and that certain substitution patterns favor inhibition of Gram-negative bacteria.

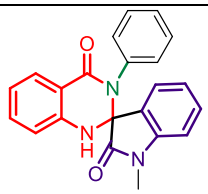
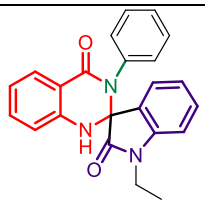
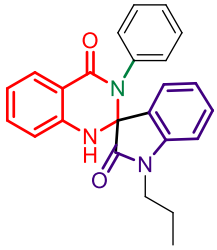
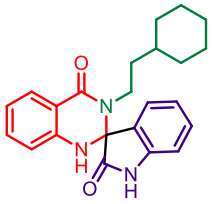
3.3 Activity Against *Pseudomonas aeruginosa* (ATCC BAA-427)

None of the synthesized compounds showed significant inhibition of *P. aeruginosa*, and all derivatives exhibited IC_{50} values greater than 50 µM. This lack of activity is not unexpected, as *P. aeruginosa* possesses a highly impermeable outer membrane, multiple efflux pumps, and intrinsic resistance mechanisms that reduce susceptibility to many small molecules. The uniformly poor activity against this strain underscores the formidable challenge associated with designing compounds capable of overcoming the defense mechanisms of this pathogen.

Table 1: Antibacterial activity of the synthesized quinazolinones and spiroquinazolines

Antibacterial Activity (MIC in µm against)					
S. No.	**Comp. No.**	**Structure**	***E. Coli* (ATCC 9637)**	***P. aeruginosa* (ATCC BAA-427)**	***S. aureus* (ATCC 25923)**
1	4a		**50**	>50	>50
2	4b		**50**	>50	**1.56**
3	4c		>50	>50	>50
4	4d		>50	>50	>50
5	4e		>50	>50	>50

6	4f		>50	>50	>50
7	4g		>50	>50	>50
8	4h		>50	>50	>50
9	4i		**12.5**	>50	**1.56**
10	6a		**12.5**	>50	>50
11	6b		**12.5**	>50	>50

12	6c		>50	>50	>50
13	6d		**12.5**	>50	>50
14	6e		**12.5**	>50	>50
15	6f		**12.5**	>50	>50

3.4 Activity Against *Staphylococcus aureus* (ATCC 25923)

The most significant biological results were obtained against the Gram-positive strain *S. aureus*. Two quinazolinone derivatives, 4b and 4i, exhibited potent antibacterial activity with IC_{50} values of 1.56 µM. This level of inhibition is notable and identifies these compounds as the most promising members of the synthesized library. Compound 4b contains a para-methoxy-substituted aromatic ring, while compound 4i incorporates tyramine as the amine component. The high potency of these derivatives suggests that both electronic effects and the presence of a hydroxyethylphenyl side chain contribute favorably to interactions with the bacterial target. All remaining compounds in the series were inactive against *S. aureus* (IC_{50} > 50 µM), emphasizing the importance of precise structural requirements for potent antibacterial action.

3.5 Comparative Analysis of Quinazolinone and Spiroquinazolinone Scaffolds

Comparison of the two structural classes revealed distinct activity profiles. The classical quinazolinone derivatives produced the strongest activity against *S. aureus*, while the spiroquinazolinones were more active against *E. coli*. The rigid spiro architecture may enhance three-dimensional complementarity with targets in Gram-

negative bacteria, whereas the planar quinazolinone framework appears better suited for potent inhibition of Gram-positive organisms. The results demonstrate that scaffold topology has a significant influence on bacterial selectivity. Introduction of a spiro center altered the spectrum of activity rather than uniformly improving potency, highlighting the importance of conformational effects in medicinal chemistry optimization.

3.6 Preliminary Structure–Activity Relationship (SAR)

The antibacterial screening provided several useful structure–activity insights:

1. **Para-methoxy substitution enhanced potency.** Compound 4b containing a 4-methoxy group exhibited excellent activity against *S. aureus*.
2. **Tyramine-derived side chains improved biological activity.** Compound 4i was active against both *E. coli* and *S. aureus*, suggesting that the phenolic hydroxyethyl substituent contributes to favorable target interactions.
3. **Multiple methoxy substituents were detrimental.** Increasing substitution, as observed in related analogues, led to a marked loss of activity.
4. **Spirocyclization altered bacterial selectivity.** Spiroquinazolinones generally retained moderate activity against *E. coli* but did not show significant potency against *S. aureus*.
5. **Substitution on the spiro aromatic ring often reduced activity.** Unsubstituted or minimally substituted spiro derivatives performed better than heavily substituted analogues.

The present study demonstrates that quinazolinone-based heterocycles synthesized through an environmentally friendly surfactant-mediated approach possess promising antibacterial properties. Although the majority of compounds were inactive, a small number displayed highly selective and potent inhibition, particularly against *S. aureus*. The identification of compounds 4b and 4i with IC_{50} values of 1.56 μM highlights the potential of this scaffold for further optimization. These findings are especially encouraging because the compounds were synthesized using a green and operationally simple methodology, allowing rapid generation of structurally diverse analogues. The combination of sustainable synthesis and meaningful biological activity provides a strong foundation for future medicinal chemistry efforts aimed at developing new antibacterial agents based on the quinazolinone pharmacophore.

4. Conclusion

In the present study, a focused library of quinazolinone and spiroquinazolinone derivatives was successfully evaluated for antibacterial activity against three clinically relevant bacterial strains, namely *Escherichia coli* (ATCC 9637), *Pseudomonas aeruginosa* (ATCC BAA-427), and *Staphylococcus aureus* (ATCC 25923). The compounds were synthesized through an environmentally benign multicomponent protocol employing isatoic anhydride, primary amines, and substituted benzaldehydes or isatin derivatives in aqueous medium using Triton X-100 as a surfactant catalyst. This strategy provided an efficient and sustainable route to structurally diverse heterocyclic scaffolds of medicinal relevance. Biological screening demonstrated that the synthesized molecules possess selective antibacterial properties. Among the fifteen compounds tested, quinazolinone derivatives 4b and 4i emerged as the most potent analogues, exhibiting excellent activity against *Staphylococcus aureus* with IC_{50} values of 1.56 μM. Compound 4i also displayed moderate inhibition of *Escherichia coli* (IC_{50} = 12.5 μM), indicating a broader antibacterial spectrum. Several spiroquinazolinone derivatives (6a, 6b, and 6d–6f) showed moderate activity against *E. coli*, while none of the compounds

demonstrated significant activity against *Pseudomonas aeruginosa*, a pathogen well known for its intrinsic multidrug resistance.

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Conflict of Interest

Authors declare no conflict of interest.

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