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ORIGINAL RESEARCH ARTICLE

Evalution of the Antibacterial Potential of a Newly Synthesized (5aR,11aS,11bR)- spiro Tetraone Against E. coli and S. aureus

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Abstract

Background Antibiotic resistance among pathogenic bacteria represents a critical global health concern, underscoring the urgent need for novel antibacterial agents. The compound (5aR,11aS,11bR)-1,2,3,5a,11a,11b-hexahydrospiro[benzo[f]pyrrolo[2,1-a]isoindole-5,2'-indene]-1',3',6,11-tetraone (compound 6) and its derivatives constitute a new class of potential antibacterial agents. This study aimed to evaluate the antibacterial activity of the synthesized compound 6 against resistant bacterial strains.

Methods Compound 6 was synthesized via a one-pot 1,3-dipolar cycloaddition reaction involving azomethine ylide, generated in situ from ninhydrin, 1,4-naphthoquinone, and proline, using ethanol as an environmentally friendly solvent. The molecular structure and functional groups of the synthesized compound were characterized by Fourier-transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (^1H NMR) analysis. Antibacterial activity was evaluated by determining the minimum inhibitory concentration and minimum bactericidal concentration against standard strains of Escherichia coli and Staphylococcus aureus using broth microdilution and agar culture methods.

Results FT-IR and ^1H NMR spectral analyses confirmed the purity and regioselectivity of the synthesized product. Antibacterial testing revealed that compound 6 exhibited minimum inhibitory concentration and minimum bactericidal concentration values of 2.5 µg/mL for both S. aureus and E. coli. The minimum inhibitory concentration and minimum bactericidal concentration values of the standard antibiotics were 0.3 µg/mL and 1.2 µg/mL for gentamicin (against E. coli), and 1 µg/mL for vancomycin (against S. aureus).

Conclusion Compound 6 demonstrated notable antibacterial activity against Escherichia coli and Staphylococcus aureus, with minimum inhibitory concentration and minimum bactericidal concentration values comparable to those of standard antibiotics. The compound exhibited a stronger bactericidal effect against the Gram-positive bacterium S. aureus than against the Gram-negative E. coli. Further in vitro and in vivo studies are warranted to evaluate its safety profile and potential therapeutic applications.

Keywords 1,3 dipole ring formation, Anti-Bacterial agents, Escherichia coli, Pyrrolizidine, Staphylococcus aureus

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1 Introduction

The discovery of antibiotics in the twentieth century marked a major breakthrough in the fight against bacterial infections, revolutionizing modern medicine and significantly extending human life expectancy. However, the rapid emergence of antibiotic resistance now threatens these achievements, as bacteria increasingly develop mechanisms to evade existing therapies. Multidrug resistance among pathogens has therefore become a critical global health concern requiring urgent intervention.[1]

Antimicrobial resistance (AMR), often referred to as the "silent pandemic," demands immediate and coordinated global action rather than being regarded as a distant threat. Without effective preventive measures, AMR is projected to become the leading cause of death worldwide by 2050. In 2019, nearly 5 million deaths were directly associated with AMR, and in the United States alone, more than 2.8 million antimicrobial-resistant infections are reported annually. Without sustained control efforts, global mortality attributable to AMR could rise to approximately 10 million deaths per year by 2050.[2,3]

Staphylococcus aureus isolates obtained from clinical specimens worldwide are exhibiting increasing resistance to a wide spectrum of antimicrobial agents, leaving few effective bactericidal options for treating these potentially fatal infections.[4] The global prevalence of antibiotic-resistant S. aureus, particularly methicillin-resistant S. aureus (MRSA), has reached epidemic proportions, affecting both hospital and community settings.[5] The World Health Organization (WHO) has classified MRSA as a Priority 2 (High) pathogen on its global list of antibiotic-resistant bacteria.[6]

Similarly, the rise of multidrug-resistant Escherichia coli has been documented in many regions over recent decades, posing a significant challenge to infection management. Plasmid-mediated extended-spectrum β-lactamase (ESBL)-producing E. coli strains are now widespread globally and display resistance to β-lactam antibiotics.[7] E. coli can acquire antibiotic resistance through multiple mechanisms, including enzymatic inactivation or modification of drugs, alteration of binding targets, active efflux of antibiotics, and metabolic adaptations that reduce susceptibility.[8]

Pyrrolizidine alkaloids have attracted considerable attention in medicinal and organic chemistry due to their structural diversity and pharmacological potential. These compounds, naturally produced by plants as defense agents against herbivores, exhibit diverse biological activities. Likewise, 2-substituted-1,3-indandiones are known for their broad pharmacological properties, including anti-complement, anti-inflammatory, anticoagulant, antitussive, and analgesic effects. Incorporating a pyrrolizidine ring at the 2-position of 1,3-indandiones is hypothesized to enhance these biological activities.[9-20]

Accordingly, this study aimed to evaluate the antibacterial activity of the newly synthesized compound (5aR,11aS,11bR)-1,2,3,5a,11a,11b-hexahydrospiro[benzo[f]pyrrolo[2,1-a]isoindole-5,2'-indene]-1',3',6,11-tetraone (compound 6) against reference strains of Staphylococcus aureus and Escherichia coli, representing Gram-positive and Gram-negative bacteria, respectively.

2 Methods

Reagents, Culture Media, and Microbial Standard Strains

Mueller–Hinton Broth (MHB) and Mueller–Hinton Agar (MHA) media were obtained from Merck (Darmstadt, Germany). Standard antibiotic powders, including vancomycin and gentamicin, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 29313 strains were provided by the Pasteur Institute of Iran.

General Chemistry

All starting materials, reagents, and solvents were procured from Merck and Aldrich and used without further purification. Reaction progress and product purity were monitored by thin-layer chromatography (TLC) using silica gel 250 μm F254 plastic sheets. The uncorrected melting points of the final derivatives were determined using a Thermo Scientific A9300 apparatus in accordance with the manufacturer's instructions.

Infrared (IR) spectra were recorded on a PerkinElmer Spectrum spectrometer using KBr disks. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 500 DRX Advance spectrometer (tetramethylsilane [TMS] as the internal standard). Chemical shifts (δ) were reported in parts per million (ppm), downfield from TMS. Proton coupling patterns were expressed as multiplet (m), doublet (d), and triplet of doublets (td). Mass spectra were obtained using a Shimadzu QP-1100 EX mass spectrometer operating at 70 eV ionization potential. Elemental analyses for C, H, and N were conducted using a Herase CHN–O Rapid analyzer. Microwave irradiation was carried out in a Daewoo DC oven (2450 MHz, 800 W).

Synthesis of (5aR,11aS,11bR)-1,2,3,5a,11a,11b-Hexahydrospiro[benzo[f]pyrrolo[2,1-a]isoindole-5,2'-indene]-1',3',6,11-tetraone (ZCompound 6) Under Microwave Irradiation

In a 50 mL Erlenmeyer flask, a mixture of ninhydrin 1 (0.178 g, 1 mmol), proline 2 (0.115 g, 1 mmol), and 1,4-naphthoquinone 5 (0.158 g, 1 mmol) was dissolved in absolute ethanol (8 mL). The reaction

mixture was irradiated in a microwave oven (800 W) for 2 minutes. During the reaction, the mixture boiled vigorously, liberating CO_2 gas (this gas evolution should be considered). Reaction progress was monitored by TLC. Upon completion, the solvent was evaporated under reduced pressure to afford light green crystals of (5aR,11aS,11bR)-1,2,3,5a,11a,11b-hexahydrospiro[benzo[f]pyrrolo[2,1-a]isoindole-5,2'-indene]-1',3',6,11-tetraone (compound 6) in 89% yield (m.p. 167 °C, ethanol). (Figure 1).[13]

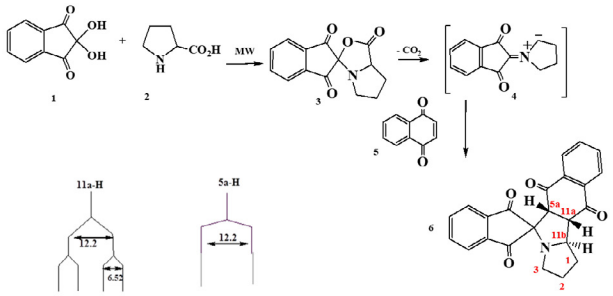

Figure 1 Synthesis of the new compound 6

Spectroscopic data:

The structures of the obtained compounds were verified using spectroscopy (Mass, IR, 1HNMR) and elemental analysis (Figure 2, Figure 3 and Figure 4). Spectral data of (compound 6) are provided below:

1H NMR (acetone-d_6, 500 MHz): δ 1.71–1.80 (1H, m, 7'-H), 2.24–2.31 (1H, m, 7'-H), 2.57–2.65 (1H, m, 6'-H), 2.69–2.77 (1H, m, 6'-H), 2.80–2.94 (1H, m, 5'-H), 2.96–3.04 (1H, m, 5'-H), 3.70 (1H, dd, J = 6.5, 12.3 Hz, 1'-H), 4.28 (1H, td, J = 3.5, 7.4 Hz, 7'a-H), 4.50 (1H, d, J = 12.3 Hz, 2'-H), 7.73–8.16 (8H, m, ArH).

IR (υ_max/cm^{-1}, KBr): 1660 (C=O), 1700 (C=O).

MS (m/z, %): 371 (M^+, 15), 213 (M^+−158, 15), 104 (158−2CO, 50).

Anal. Calcd. for $C_{23}H_{17}NO_4$ (M.W. = 371.38): C, 74.38; H, 4.61; N, 3.77; O, 17.23%. Found: C, 74.38; H, 4.62; N, 3.77; O, 17.25%.

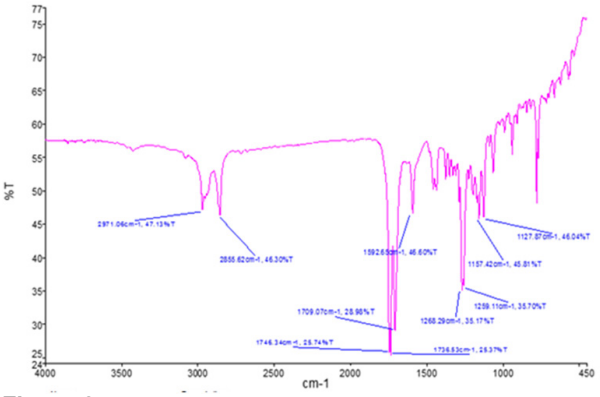

Figure 2 IR (cm-1) spectrum of compound 6

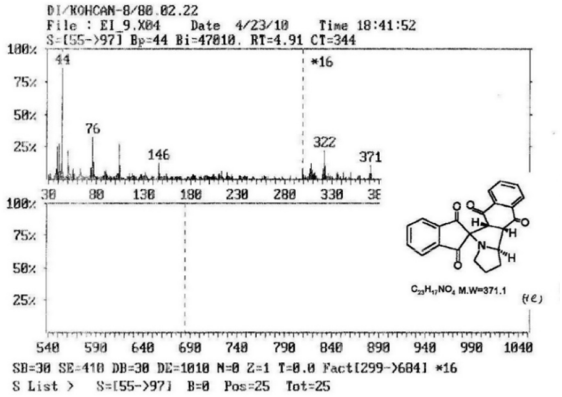

Figure 3 Mass spectrum of compound 6

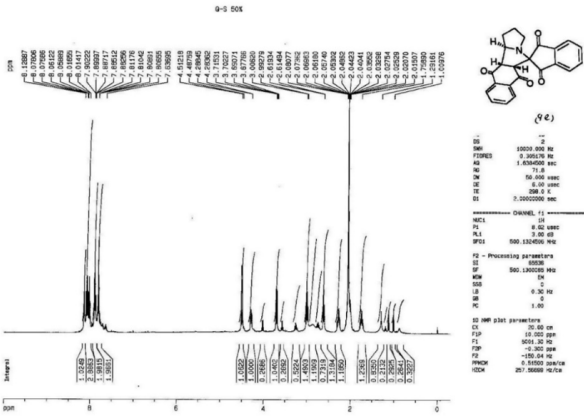

Figure 4 H-NMR spectrum of a novel spiro indene-pyrrolizidine alkaloid 6

Preparation of Bacterial Strains

Reference bacterial strains E. coli ATCC 25922 and S. aureus ATCC 29313 were used in this study. According to the standard microbiology manual, frozen bacterial stocks were thawed and cultured in trypticase soy broth (TSB) at 37 °C for 24 hours. A 100 µL aliquot of each culture was then inoculated onto Mueller–Hinton agar and incubated under the same conditions. Working stocks were maintained on MHA plates and stored at 4 °C for future use.

Antibacterial Assay

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of compound 6 and standard antibiotics was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines using the broth microdilution method.[21] Briefly, 100 µL of sterile MHB was added to each well of a 96-well microplate. Serial twofold dilutions of compound 6 and standard antibiotics were prepared to achieve final concentrations ranging from 5 µg/mL to 0.039 µg/mL.

Bacterial suspensions were adjusted to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and measured

spectrophotometrically at 625 nm. Subsequently, 100 µL of each adjusted bacterial suspension (final inoculum: 5×10^5 CFU/mL) was added to each well. Plates were incubated at 37 °C for 16–20 hours.

MIC values were determined by visual inspection and confirmed by measuring absorbance at 625 nm using a microplate reader (Epoch, BioTek Instruments, Winooski, VT, USA). Sterile MHB served as the negative control, and bacterial suspension in MHB without antibiotics was used as the positive control. All assays were performed in duplicate.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined in accordance with CLSI guidelines.[19] Following MIC determination, 10 µL from each well showing no visible growth was plated onto Mueller–Hinton agar and incubated at 37 °C for 24 hours. The MBC was defined as the lowest concentration of compound 6 or standard antibiotic that resulted in ≥99.99% reduction in viable bacterial colonies.

3 Results

Antibacterial Assay

Minimum Inhibitory Concentration (MIC)

The MIC values of the newly synthesized compound 6 and reference antibiotics were determined against Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 29313. For E. coli, the MICs of compound 6 and gentamicin were 2.5 µg/mL and 3.2 µg/mL, respectively. For S. aureus, compound 6 and vancomycin inhibited bacterial growth at concentrations of approximately 2.5 µg/mL and 1 µg/mL, respectively (Table 1).

Table 1 MIC results for compound 6 and the antibiotics gentamicin and vancomycin against E.

Strains	Synthetic compounds	Gentamycin	Vancomycin
	MIC ± SD (µg/ml)	MIC ± SD (µg /ml)	MIC ± SD (µg /ml)
E. coli ATCC 25922	2.5±0.0	0.3±0.0	-
S. aureus ATCC 29313	2.5±0.0	-	1±0.0

E.coli and S. aureus.Vancomycin against E. coli and S. aureus.

Minimum Bactericidal Concentration (MBC)

The MBC values for compound 6 and standard antibiotics are summarized in Table 2. Against E. coli ATCC 25922, compound 6 and gentamicin showed MBCs of 2.5 µg/mL and 6.4 µg/mL, respectively. At 2.5 µg/mL, compound 6 eradicated more than 99% of E. coli cells. For S. aureus ATCC 29313, both compound 6 and vancomycin were bactericidal at concentrations of 2.5 µg/mL and 0.2 µg/mL, respectively, with compound 6 eliminating over 99% of bacterial cells at 2.5 µg/mL (Table 2, Figure 5, and Figure 6).

Table 2 MBC results for compound 6 and reference antibiotics against E. coli and S. aureus.

Strains	Synthetic compounds 6	Gentamycin	Vancomycin
	MBC ± SD (µg /ml)	MBC ± SD (µg /ml)	MBC ± SD (µg /ml)
E. coli ATCC 25922	2.5±0.0	1.2±0.0	-
S. aureus ATCC 29313	2.5±0.0	-	1±0.0

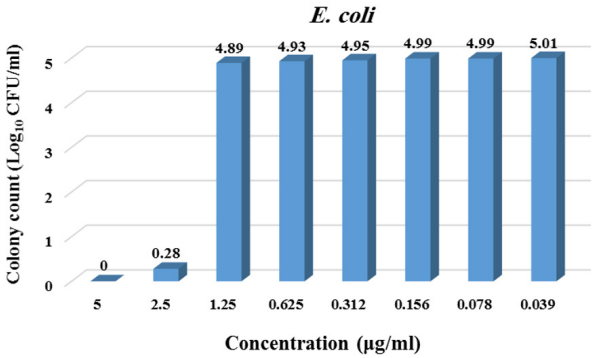

Figure 5 MBC of the compounds 6 against E. coli was assessed. At a concentration of 5 µg/ml, the compounds 6 completely killed all E. coli cells, whereas at 2.5 µg/ml, they eliminated more than 99% of the cells

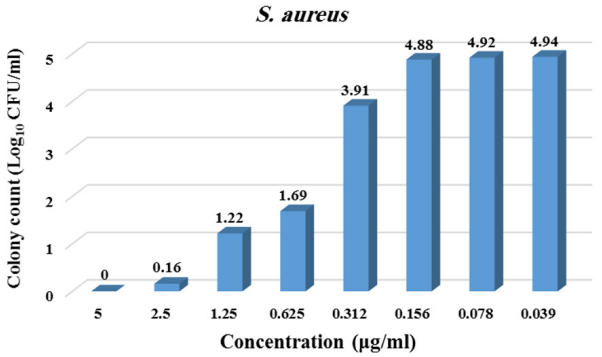

Figure 6 MBC of the compounds 6 against *S.aureus* was assessed. At a concentration of 5 µg/ml, the compounds 6 completely killed all *S.aureus* cells, whereas at 2.5 µg/ml, they eliminated more than 99% of the cells.

4 Discussion

The increasing prevalence of antibiotic-resistant bacteria has created an urgent demand for novel antimicrobial agents with improved efficacy and broader activity spectra. Among the promising structural scaffolds, pyrrolizidine alkaloids, a class of naturally occurring heterocyclic secondary metabolites, have exhibited diverse pharmacological effects, including significant antibacterial activity.[13] In the present study, a novel compound containing a fused pyrrolizidine ring system was synthesized via a one-step azomethine ylide cycloaddition reaction employing α-amino acids and ninhydrin. The resulting compound, (5aR,11aS,11bR)-1,2,3,5a,11a,11b-hexahydrospiro[benzo[f]pyrrolo[2,1-a]isoindole-5,2'-indene]-1',3',6,11-tetraone (compound 6), was hypothesized to possess potent antibacterial activity due to its unique structural characteristics.

Pyrrolizidine derivatives are known to interfere with bacterial growth by targeting key components of the cell envelope and metabolic pathways. In this study, compound 6 demonstrated inhibitory and bactericidal activity against both Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive). The compound completely eradicated both bacterial species at a concentration of 5 μg/mL, and retained strong bactericidal activity at 2.5 μg/mL, eliminating over 99% of bacterial cells. These findings indicate that compound 6 is effective at relatively low concentrations against representative strains of both Gram types.

The MIC and MBC assays revealed that for E. coli, compound 6 exhibited both MIC and MBC values of 2.5 μg/mL, which were lower than those of gentamicin (MIC = 3.2 μg/mL; MBC = 6.4 μg/mL). This suggests a stronger antibacterial potency for compound 6 and the potential for achieving bactericidal activity at lower concentrations, which may reduce toxicity risks and slow resistance development. The equality of MIC and MBC values further supports a bactericidal mode of action, a desirable characteristic in antimicrobial drug design.

Against S. aureus, compound 6 exhibited MIC and MBC values of 2.5 μg/mL, whereas vancomycin showed higher potency (MIC = MBC = 0.2 μg/mL). Nevertheless, at equivalent concentrations, compound 6 demonstrated more pronounced bactericidal effects against S. aureus than E. coli, as reflected by fewer surviving colonies. This difference may be attributed to more efficient interactions between the compound and the thick peptidoglycan layer of Gram-positive bacteria.

Although vancomycin remains more potent, the comparable performance of compound 6 highlights its potential as a structural scaffold for developing new broad-spectrum antibacterial agents. Moreover, the equivalence of MIC and MBC values in both tested bacteria implies that compound 6 can both inhibit growth and cause bacterial death at the same concentration, minimizing the need for higher doses that might induce side effects or resistance.

The observed activity aligns with previous reports suggesting that pyrrolizidine alkaloids exert antibacterial effects primarily through disruption of the bacterial cell membrane.[22] The higher susceptibility of S. aureus may be related to differences in the cell wall structure between Gram-positive and Gram-negative species; the outer membrane of E. coli may restrict compound penetration, slightly reducing its killing efficiency.

The results are consistent with prior studies. For example, Supo et al. (2022) synthesized two pyrrolidine-2,5-dione derivatives fused to a dibenzobarrelene backbone, which exhibited MIC values ranging from 16 to 256 μg/mL, notably higher than those observed for compound 6, indicating its superior activity.[23] Similarly, Sirisha et al. (2013) synthesized a series of dispiro pyrrolidines through cycloaddition of azomethine ylides, which showed good antimicrobial activity against several human pathogens.[24] Akbari et al. (2024) reported the synthesis of methyl 2'-methyl-1,3-dioxo-1,1',2',3,5',6',7',7a'-octahydrospiro[indene-2,3'-pyrrolizidine]-2'-carboxylate with MIC and MBC values of 12.5 μg/mL against both E. coli and S. aureus [25], further confirming that the compound reported in this study displays greater antibacterial potency.

Taken together, these findings demonstrate that the synthesized pyrrolizidine-based compound exhibits broad and potent antibacterial activity against both Gram-positive and Gram-negative bacteria. Its promising efficacy, simple synthetic route, and structural novelty make it a valuable lead compound for further pharmacological optimization and mechanistic studies aimed at developing next-generation antimicrobial agents.

5 Conclusion

Compound 6 demonstrated significant broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. The compound exhibited MIC and MBC values of 2.5 μg/mL against Escherichia coli and Staphylococcus aureus, confirming its potent bactericidal effect. Although slightly less active than standard antibiotics, its antimicrobial potency remains highly promising.

Overall, the low MIC and MBC values highlight compound 6 as a potential lead candidate for antibacterial drug development. Further studies, including cytotoxicity, pharmacokinetic evaluation, and assessment against multidrug-resistant strains, are necessary to confirm its therapeutic applicability.

Compound 6, synthesized through the original formulation, demonstrated broad-spectrum antibacterial activity, effectively inhibiting the growth of both Gram-positive and Gram-negative bacteria at a single concentration. A dosage twice the MIC value was sufficient to completely eliminate bacterial growth. The compound exhibited MIC and MBC values of 2.5 μg/mL against Escherichia coli, indicating strong antimicrobial potential that is comparable to the MBC of gentamicin (1.2 μg/mL). Similarly, compound 6 displayed MIC and MBC values of 2.5 μg/mL against Staphylococcus aureus, which, although slightly less potent than vancomycin (MIC = MBC = 1 μg/mL), still reflects a promising antibacterial profile.

Overall, the synthesized compound exhibits potent bactericidal activity against both E. coli and S. aureus, with particularly enhanced effects against the Gram-positive strain. Its relatively low MIC and MBC values, combined with possible membrane-disruptive or enzyme-inhibitory mechanisms, suggest that it could serve as a promising lead molecule for new antibacterial drug development. Further investigations, especially regarding its pharmacokinetic profile, cytotoxicity, and efficacy against multi-drug-resistant strains, are essential. In addition, in vitro and in vivo toxicity assessments are needed to confirm its safety and therapeutic potential before clinical application.

Declarations

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Artificial Intelligence Disclosure
Artificial intelligence has been used for literary editing.

Authors' Contributions
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

Availability of Data and Materials
Data are available on request from the authors.

Conflict of Interest
None of the authors have any conflict of interest to declar.

Consent for Publication
Not applicable.

Ethical Considerations
This article is derived from the first author's PhD thesis in Pharmacy, which was approved by the Research Council of Urmia University of Medical Sciences and is acknowledged here by citing the source (http://ethics.research.ac.ir/IR.UMSU.REC.1402.145).

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