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ORIGINAL ARTICLE

Green Synthesis of Benzoxazolone/Benzothiazolone based Thiazole and Oxazole Analogues: Antimicrobial Evaluation and Molecular Docking Study for Sustainable Drug Discovery

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ABSTRACT
The emergence of antibiotic resistance necessitates the discovery of novel antimicrobial agents. Benzoxazolone and benzothiazolone derivatives possess promising antimicrobial properties. However, traditional synthesis methods often involve hazardous chemicals and generate harmful waste. This study aimed to develop a green synthesis approach for benzoxazolone/ based Thiazole and Oxazole analogues, evaluate their antimicrobial activity, and utilize molecular docking to explore their potential as drug candidates. This research employed a green synthesis method i.e. ultrasonication to synthesize benzoxazolone and benzothiazolone analogues. Subsequently, the antimicrobial activity of the synthesized compounds against various pathogens was assessed. Additionally, molecular docking studies were performed to investigate the binding interactions between the compounds and the target protein of the E coli bacteria. The study successfully established a sustainable method for synthesizing the desired analogues. The synthesized compounds exhibited significant antimicrobial activity against a range of tested pathogens. Molecular docking analysis revealed promising binding interactions between the compounds and the target protein, suggesting their potential as antimicrobial agents. This research demonstrates a green and sustainable approach for synthesizing potential antimicrobial drug candidates. The synthesized benzoxazolone/benzothiazolone based Thiazole and Oxazole analogues displayed promising antimicrobial activity and favorable interactions with target protein during in-silico analysis. Further investigations are crucial to evaluate their in-vivo efficacy and potential toxicity.
Keywords: *Green synthesis, Benzoxazolone/Benzothiazolone based Thiazole and Oxazole analogues, Antimicrobial activity, Molecular docking, Drug discovery.*

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INTRODUCTION

The emergence of multidrug-resistant (MDR) pathogens poses a significant threat to global health [1, 2]. Discovering new and effective antimicrobial agents is crucial to combat this challenge [3, 4]. Benzoxazolone and benzothiazolone derivatives hold promise as novel antimicrobial agents due to their diverse biological activities [5-8, 35]. However, traditional synthetic methods often employ harsh chemicals and generate hazardous waste often raise environmental and safety concerns [9, 10]. Exploring green synthesis methods is not just an environmental imperative. But also a safety and ethical necessity for developing new antimicrobial agents. Green chemistry offers a more sustainable and responsible solution for drug discovery, by minimizing environmental impact, enhancing safety, and promoting public trust, green chemistry paves the way for the development of safer and more efficacious drugs, including those derived from promising scaffolds like benzoxazolone and benzothiazolone [9,11-13]. Benzoxazolone and benzothiazolone are two closely related heterocyclic compounds containing a five-membered aromatic ring fused with either an oxazole or thiazole ring, respectively. These scaffolds hold significant biological importance due to their diverse range of activities, making them valuable candidates for drug discovery and development [14-17].

Benzoxazolone: This five-membered ring structure consists of one nitrogen atom and one oxygen atom, fused with a benzene ring [18]. The general formula for benzoxazolone is $C_6H_5NO_2$ [Figure 1].

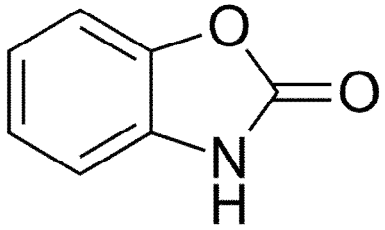


Figure 1: Structure of Benzoxazolone.

Benzothiazolone: Similar to benzoxazolone, it also has a five-membered ring but with one nitrogen atom and one sulfur atom, again fused with a benzene ring [19]. The general formula for benzothiazolone is C_6H_5NSO [Figure 2].

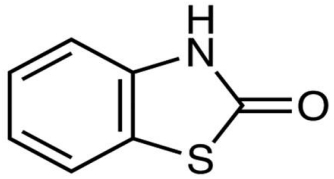


Figure 2: Structure of benzothiazolone.

These Benzoxazolone and benzothiazolone compounds exhibit a wide range of biological activities, making them promising candidates for various applications. Several studies have demonstrated the effectiveness of these compounds against various bacteria, fungi, and plant viruses [20-23]. Certain derivatives have shown promising antitumor properties, potentially inhibiting cancer cell proliferation and migration [24]. These compounds can scavenge free radicals and reactive oxygen species, potentially protecting cells from oxidative damage [25]. Some derivatives exhibit anti-inflammatory properties, potentially alleviating symptoms associated with inflammatory diseases [17]. These scaffolds are also explored for their potential in treating neurodegenerative diseases, diabetes, and various other conditions.

This study focuses on the green synthesis of benzoxazolone and benzothiazolone analogues, followed by evaluation of their antimicrobial activity and a molecular docking study. This research study contributes to sustainable drug discovery by implementing environmentally friendly synthesis methods, investigating promising antimicrobial candidates and employing computational tools to optimize drug design. By combining these elements, the study paves the way for the development of novel and effective antimicrobial agents in a more sustainable and responsible manner.

MATERIALS AND METHODS

Compounds used in this study are purchased from Lotus Ltd used as received. All the solvents were of analytical grade and were distilled before used. The solid IR (spectra were recorded in KBr on Bruker-ALPHA-II spectrophotometer at GITAM University, Visakhapatnam; however, 1H- NMR and 13C-NMR (spectra were obtained using Bruker 400 MHz NMR facility at Andhra University Visakhapatnam.) Chemical shift values were reported in parts per million (ppm) using TMS as an internal standard and DMSO-d6/CDCl3 as NMR solvents. Ultra-sonication (US) experiments were performed on a probe sonicator (12/20 mm probe) with an ultrasonic processor (250W) operating at a fixed frequency of 50 Hz with speed 238 rpm. Quantum chemical calculations were performed using Gaussian 16 [26] at B3LYP/6-31G (d) level of theory [27-29]. Positive frequencies showed that all the optimized structures were minimal. The mechanistic study was carried out with the free energy of each of the intermediates.

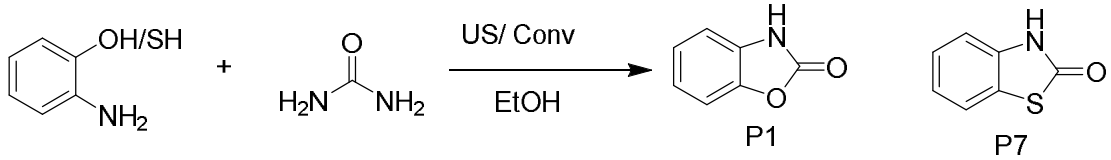
The synthesized compounds (P1-P12) were examined for antimicrobial assay against three bacteria [Gram negative: EC = *Escherichia coli* and KP = *Klebsiella pneumonia]* and [Gram positive: SA=*Staphylococcus aureus*] using the disc diffusion method [30] at the department of Biotechnology, GSS GITAM. 200 mL of nutrient agar growth medium was dispensed into sterile conical flasks, these were then

inoculated with 20µL of cultures mixed gently and poured into a sterile Petri dish. After setting a borer with 6 mm diameter was properly sterilized by flaming and used to make four uniform wells in each Petri dish. The wells were loaded with 50µL of different investigated compounds under study. The solvent DMSO, used for reconstituting solvent for diluting the compounds were similarly analyzed for control. During the experiment amoxicillin is used as control. The plates were incubated at 37^0C for 24 h. The zone of inhibition (mm) was measured with a Hi Anti Biotic Zone Scale in mm and the experiment was carried out in triplicate experiments. The values were averaged and are presented. Docking analysis was performed with the optimized structures and the 6NTW PDB structure of the *E. coli* downloaded from the RCSC PDB [31] using the AUTODOCK VINA software [32,33]. The BIOVIA Discovery Studio visualization [34] was used for the 3D and 2D images of the docking analysis of the different molecules.

RESULTS AND DISCUSSION

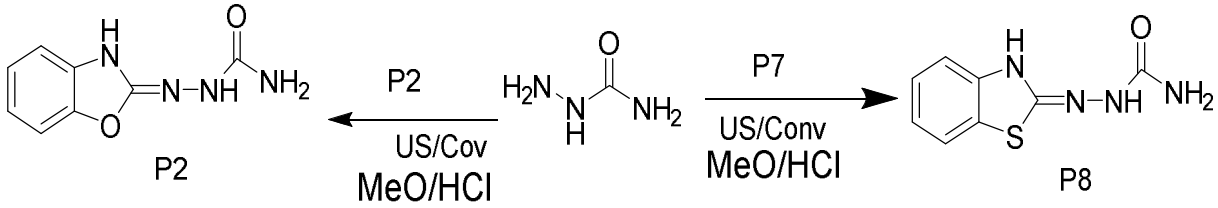
Synthesis and Characterization

Benzoxazolone and benzothiazolone are versatile compounds and they have several biological activities, it prompted us to synthesis some of their derivatives by adopting conventional and green methods. The target compounds were prepared in three steps. In the first step of synthesis Scheme 1, o-aminophenol/ o-amino thiophenol and urea were dissolved in ethanol to obtain P-1/ P-7 by adopting conventional and ultrasonication.



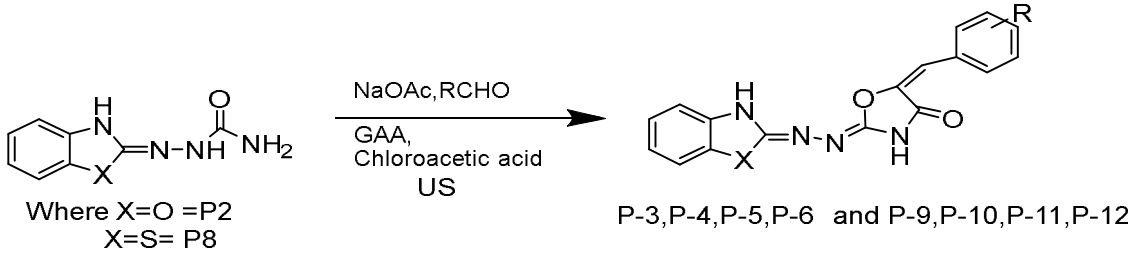
Scheme 1: Synthetic pathway for Benzoxazolone (P1) and benzothiazolone (P7).

In the second step of synthesis Scheme 2, then P-1/P-7 reacted with thiosemicarbazide and semicarbazide-hydrochloride respectively to obtain P-2/P-8.



Scheme 2: Synthetic pathway for compounds P2 and compounds P8 compound

In the 3rd step of synthesis scheme 3, P-2/P8 and substituted aldehydes (4-chlorobenzaldehyde, 4-hydroxybenzaldehyde, 4-methoxybenzaldehyde, 2,5-dimethoxybenzaldehyde) were dissolved in ethanol along with chloroacetic acid, sodium acetate, glacial acetic acid to obtain P-3, P-4,P-5,P-6 and P-9,P-10,P-11,P-12 respectively. All the three steps compounds were also synthesized by conventional synthesis. The reaction kinetics and yields were compared for both the methods. It was observed that better yields were obtained in Ultrasonication method, reducing the time of reaction and improving the yield of the reaction (Table 1). All steps compounds were well characterized by spectral mean and found consistent with expected structures of the synthesized compounds.



Scheme 3: Synthetic pathway for target compounds P-3 to P-6 and compounds P-9 to P-12

To confirm the structure of all step compounds spectral analysis were performed and established the structure. In the IR spectrum of P1 / P7 the major peaks observed at 1675 / 1682 and 3228/3235 cm^{-1} could be assigned to C=O and NH along with peaks for aromatic groups at 3050/3075 cm^{-1}. However in P-2/P-8 major peaks observed at cm^{-1} 1685 / 1692 for C=O and 3310/3345 for NH_2 group of thio-

semicarbazide apart from other major aromatic hydrogen peaks confirming the condensation of P1 / P7 with thio-semicarbazide to produce P-2/P-8.Additionally the formation of all target compounds were confirmed by the major peaks of C=O and NH peaks -1685 and 3230 cm^{-1} respectively in their IR spectrum. Which was also confirmed by the absence of CHO and NH_2 in the target compounds.

Further the NMR spectrum also confirmed the formation of all steps compounds. In the NMR spectrum of P1 / P7 the peaks at δ ppm 8.0 for NH proton and 7.27 (2H, d), 7.79(2H,d) for aromatic protons .However in the second step products NMR P-2/P-8 δ ppm 10.75/10.55 for NH; 6.20 NH2 ;6.73 -7.8 (Ar-4H) protons confirming the condensation of P1 / P7 with thio-semicarbazide to get P-2/P-8 . In the P3 NMR spectrum 12.3 and 11.8 for (NH:2NH); 7.75 (1CH of aldehydic side);7.66-7.61 (Ar-4H; aldehydic side);7.12-6.10 (Ar-4H of benzoxazole ring). The target compounds NMR also confirmed the formation of all designed compounds [Figure 3].

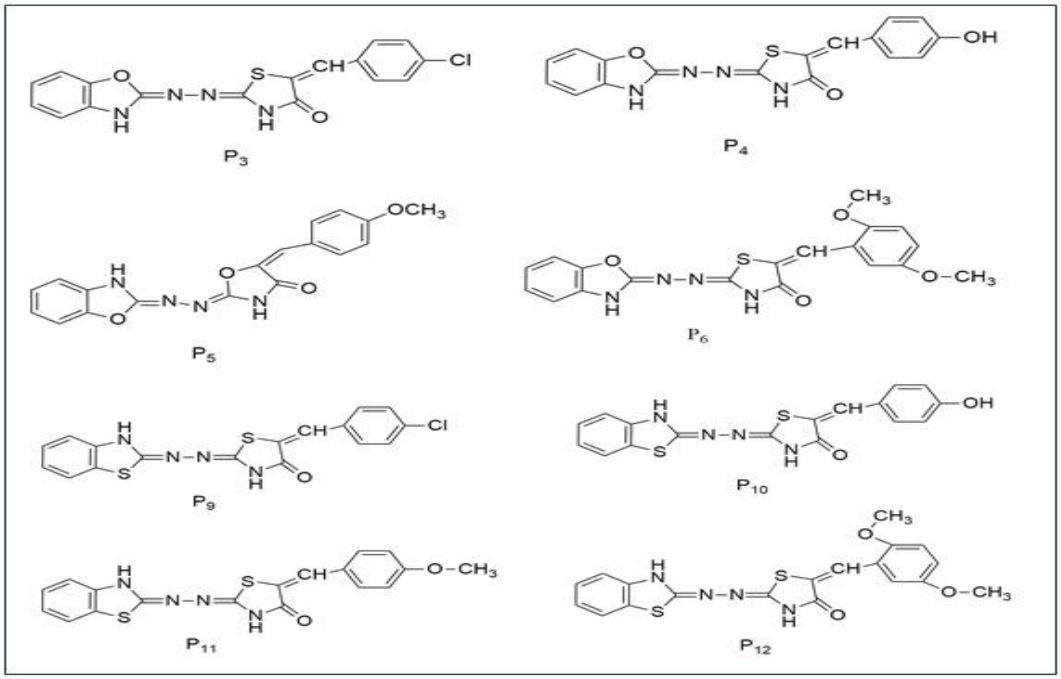


Figure 3: Structure of designed and synthesized compounds

Table 1: Comparison of reaction kinetics of conventional and ultrasonic irradiation methods for compounds P1-P12.

Compound	Conventional Reaction time	Ultrasonicator Reaction time
		Minutes (%yield)
P-1	12(78)	50min (89)
P-2	10(68)	60min (81)
P-3	16(75)	52min (91)
P-4	24(72)	52min(90)
P-5	18(71)	52min(93)
P-6	22(78)	52min (92)
P-7	8(78)	50min(96)
P-8	3(78)	50min(98)
P-9	18(76)	60min(91)
P-10	22(74)	60min(95)
P-11	16(70)	60min(88)
P-12	24(72)	60min (87)

Antibacterial activity

The results of testing twelve compounds (P1-P12) for their antimicrobial activity against various bacterial strains (Table 2). The bacteria include both gram-negative (*E. coli, K. pneumoniae*) and gram-positive (*S. aureus*) species. Several compounds (P2, P4, P6, P8, P9, P11, and P12) showed no inhibitory

effect (NA) against any of the tested bacteria. This suggests they might lack broad-spectrum antimicrobial properties. Whereas, some compounds (P1, P3, P7) exhibited activity against gram-negative bacteria (*E. coli* and *K. pneumoniae*) with inhibition zone diameters ranging from 11 mm to 23 mm. This indicates their potential effectiveness against these specific pathogens. However, only compounds P1, P3, and P7 displayed some level of activity (12-15 mm) against the gram-positive bacteria (*S. aureus*). This suggests these compounds might be less effective against this type of bacteria. The inhibition zone diameters varied among the active compounds against different bacterial strains. P7 showed the strongest and broadest activity, while P1 and P3 had a more moderate effect.

Molecular Docking Study

The results of the docking study, including the predicted binding modes and interaction patterns of the most active compounds observed and selected from antimicrobial activity against (gram negative bacteria, E coli) with the target protein with PDB ID 6NTW (Table 3).

Table 2: Antibacterial activities of synthesized compounds (10µg/ml).

Bacterial strains (in mm)				
S/N	Compound	Gram negative		Gram positive
		E. coli	*K. pneumoniae*	*S. aureus*
1	P1	15	11	12
2	P2	NA	NA	NA
3	P3	20	12	15
4	P4	NA	NA	NA
5	P5	14	NA	NA
6	P6	NA	NA	NA
7	P7	23	14	15
8	P8	NA	NA	NA
9	P9	NA	NA	NA
10	P10	12	12	NA
11	P11	NA	NA	NA
12	P12	NA	NA	NA
13	Amoxicilline	29	23	20
(NA): Indicates no anti-microbial activity				

Table 3: Molecular docking results of selected compounds from antimicrobial activity study.

S/N	**Compound**	**Docking Score (Kcal/Mol)**	**Interacting residues**
1	P1	-5.6	Leu 227, Thr 262, Pro 352, Arg 586
2	P3	**-8.1**	His 214, Gln 216, Met 378, Ser 385, Val 387, Tyr 389, Ala 583
3	P5	**-7.3**	Gln 216, Asn 380, Tyr 575, Phe 580
4	P7	-5.5	Trp 347, Val 350, Gln 354, Arg 355, Val 358, Pro 599
5	P10	**-7.6**	His 214, Pro 215, Gln 216, Val 387, Tyr 389, Ala 583

The above table lists different compounds (P1, P3, P5, P7, and P10) and their docking scores in terms of Kcal/Mol, along with the specific amino acid residues they interact with. Docking scores are crucial in the field of molecular biology and drug discovery as they predict the binding affinity between small molecule ligands (like drugs) and their target proteins. Lower (more negative) docking scores typically indicate stronger interactions between the ligand and the protein, suggesting a higher potential for the compound to act as an effective inhibitor or activator of the protein's function.

Compound P3 shows the lowest docking score of -8.1 Kcal/Mol, indicating the strongest binding affinity among the listed compounds. It interacts with a variety of residues, including His 214, Gln 216, Met 378, Ser 385, Val 387, Tyr 389, and Ala 583. The diversity and number of interacting residues might contribute to its strong binding, suggesting potential as a robust inhibitor or activator. Compound P10 also demonstrates a strong docking score of -7.6 Kcal/Mol with interactions at residues His 214, Pro 215, Gln 216, Val 387, Tyr 389, and Ala 583. Its interactions overlap significantly with P3, particularly at critical residues, which might explain its similar efficacy. Compound P5 has a docking score of -7.3 Kcal/Mol, engaging with residues Gln 216, Asn 380, Tyr 575, and Phe 580. This compound, while not binding to as

many residues as P3 or P10, still shows a substantial affinity, potentially making it a strong candidate for further investigation. Compound P1 and P7 have the highest (least negative) docking scores of -5.6 and -5.5 Kcal/Mol, respectively, indicating weaker binding affinities compared to the others. P1 interacts with residues Leu 227, Thr 262, Pro 352, and Arg 586, while P7 interacts with Trp 347, Val 350, Gln 354, Arg 355, Val 358, and Pro 599. Despite engaging with multiple residues, the interactions may not be as conducive to a strong binding affinity as those of the other compounds.

These results suggest that compounds P3, P5 and P10 have the highest potential as drug candidates due to their strong binding affinities and presented their 2D interactions (Figure 4). However, it's important to note that docking scores alone do not determine the suitability of a compound as a drug. Other factors, such as solubility, toxicity, pharmacokinetics, and pharmacodynamics, are crucial in determining a compound's viability as a therapeutic agent. Additionally, the specific function of the interacting residues and the overall structural context of the protein-ligand complex can significantly influence the biological outcome of the interaction.

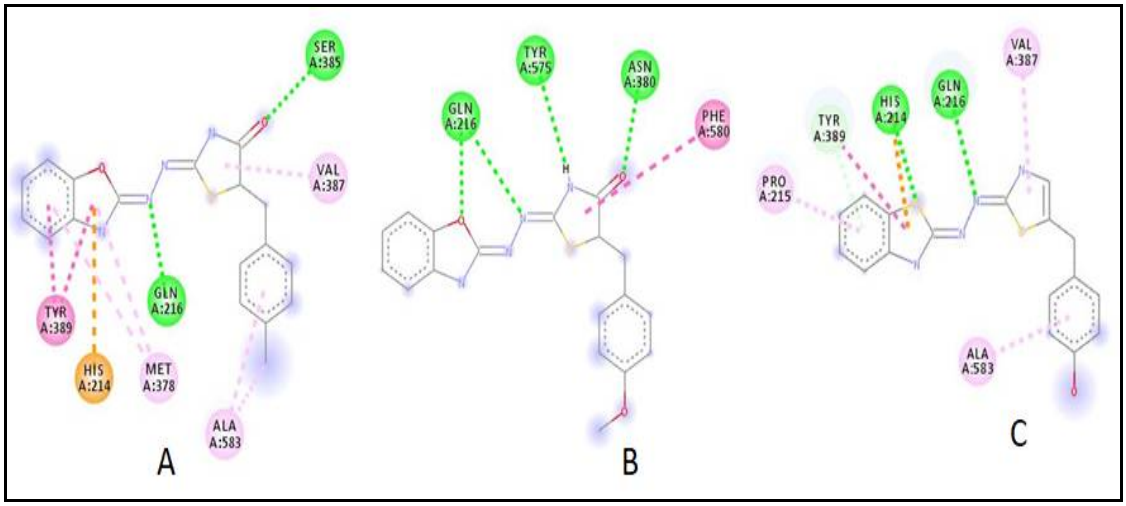


Figure 4: 2D interactions of the synthesized derivatives A) P3 B) P5 and C) P10.

CONCLUSION

The study successfully demonstrates a sustainable approach for synthesizing benzoxazolone and benzothiazolone analogues. This green synthesis method offers advantages like reduced environmental impact compared to traditional methods. The synthesized compounds exhibited promising antimicrobial activity against various pathogens especially with *E coli*. This indicates their potential as novel drug candidates. Molecular docking studies provided valuable insights into the binding interactions between the synthesized compounds and the target protein of the microbes. This information can be used for further optimization of the drug design process. Overall, the research paves the way for the development of new and effective antimicrobial agents through a sustainable and environmentally friendly approach. Further investigations are warranted to explore the in-vivo efficacy and potential toxicity of these promising candidates.

CONFLICT OF INTEREST

Declared none

FUNDING INFORMATION

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