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Research Article

Antibiotic resistance and prevalence of bacterial contaminants in street-vended suya meat in Benin City, Nigeria

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ABSTRACT

This study investigates the antibiotic resistance and prevalence of bacterial contaminants in street-vended suya meat in Benin City, Nigeria. Suya meat, a popular street food, is vulnerable to bacterial contamination due to improper handling, storage, and environmental exposure. A total of fifty (50) suya meat samples were collected from various vendors across the city for microbiological analysis. Standard microbiological methods were employed to isolate and identify bacterial pathogens, including *Bacillus* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*. The prevalence of bacterial contamination showed that 46% of samples were positive for *Escherichia coli*, 38% for *Staphylococcus aureus*, and 30% for *Pseudomonas* spp. Antibiotic susceptibility testing was performed using the disc diffusion method, revealing a high resistance rate, particularly among *E. coli* (70%), *Klebsiella* spp. (60%), and *Pseudomonas* spp. (55%) against ampicillin and tetracycline. *Salmonella* spp. displayed resistance to ampicillin (50%) and ciprofloxacin (40%). The analysis showed that *Staphylococcus aureus* was resistant to penicillin (50%) and clindamycin (45%). Statistical analysis conducted with SPSS version 23 revealed significant differences in antibiotic resistance patterns across bacterial species ($p < 0.05$). The results showed high resistance to Pefloxacin, Gentamycin, and Cotrimoxazole across most bacterial species, with *Pseudomonas* and *Klebsiella* exhibiting the highest resistance rates. Statistical analysis revealed significant correlations in antibiotic resistance between certain bacterial species, notably between *Citrobacter* and *Klebsiella* ($r = 0.939$, $p = 0.0001$) and between *Pseudomonas* and *Salmonella* ($r = 0.773$, $p = 0.015$). The results showed that *E. coli* emerged as the predominant pathogen, followed by *Pseudomonas* species and *Staphylococcus aureus* as major contributors to contamination. This study underscores the public health risk posed by bacterial contamination in street-vended suya meat, emphasizing the need for improved food safety measures and regulatory oversight to mitigate foodborne infections in Nigeria.

Keywords: Antibiotic resistance, Bacterial contaminants, Foodborne pathogens, Public health, Suya meat

1. INTRODUCTION

The problem of antibiotic resistance and bacterial contamination in street-vended foods, such as suya meat, poses significant public health risks globally. Street foods are widely consumed, especially in developing countries, due to affordability and convenience. However, poor hygiene, inadequate food safety measures, and improper handling make these foods a reservoir for bacterial contaminants like *Escherichia coli* and *Staphylococcus aureus*. Antibiotic resistance among these pathogens exacerbates the challenge, as it limits treatment options for foodborne illnesses, leading to prolonged infections, increased healthcare costs, and higher mortality rates. Globally, antibiotic resistance is a growing crisis, with the World Health Organization (WHO) labeling it one of the top threats to public health. Contaminated street foods contribute to the spread of resistant bacteria, potentially transferring resistance genes across populations. Addressing this issue requires urgent international collaboration to improve food safety standards, promote responsible antibiotic use, and enhance public health awareness.

Moreover, foodborne diseases are a major global public health issue, with significant concern in developing countries, where food safety regulations and enforcement are often inadequate. In particular, street foods, such as suya—a popular Nigerian spicy grilled meat delicacy are increasingly implicated in foodborne illness outbreaks due to microbial contamination [1,2]. Suya, which is sold widely by street vendors in urban centers across Nigeria, is valued for its affordability, convenience, and distinctive taste. Despite its popularity, the preparation and handling of suya are often performed under unsanitary conditions, raising concerns about food safety and the potential health risks to consumers [3,4]. The microbiological quality of suya has been the subject of several studies, revealing the presence of various pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp. [5-8]. These bacteria are responsible for a range of gastrointestinal diseases, including diarrhea, vomiting, and food poisoning, which can be particularly severe for vulnerable populations, such as children, the elderly, and individuals with weakened immune systems [9,10]. These pathogens are often introduced during meat processing, improper handling, or storage, and their presence in suya underscores the significant health risk posed by street-vended foods. A growing concern in the context of foodborne diseases is the presence of antibiotic-resistant bacteria in suya meat. The misuse of antibiotics in livestock farming, particularly the overuse of antibiotics for growth promotion and disease prevention, has led to the emergence of multidrug-resistant (MDR) strains of bacteria [11,12]. These resistant bacteria are not only harder to treat but also pose a threat to public health as they can be transmitted to humans through the consumption of contaminated food [13]. The prevalence of antimicrobial-resistant bacteria in street-vended suya is further compounded by the cross-contamination that occurs during meat processing and handling. The spread of these bacteria from raw to cooked meat, along with improper storage and hygiene practices, significantly increases the risk of bacterial contamination. Hygiene practices among suya vendors are a key factor contributing to the contamination of the meat. Research has revealed that many suya vendors fail to implement basic hygiene measures, such as regularly washing their hands, cleaning their utensils, and storing the meat at the appropriate temperatures [14,15]. Additionally, suya meat is often exposed to environmental contaminants, such as dust, flies, and unsanitary surfaces, which further increase the likelihood of bacterial contamination. While the use of spices in suya preparation is integral to its flavor, certain spices have also been shown to harbor antimicrobial-resistant bacteria, which could further exacerbate the risk of contamination and the spread of resistance [16,17]. Spices may create an environment in which resistant bacteria can survive and proliferate, making it essential to consider their role in the overall contamination of suya meat.

The presence of antibiotic-resistant pathogens in food poses an additional challenge to public health, as infections caused by these bacteria may not respond to conventional treatments, leading to prolonged illness and increased healthcare costs. The growing issue of antimicrobial resistance (AMR) is not only a medical concern but also a societal one, as resistant strains can spread across communities, making it

increasingly difficult to control infectious diseases [18,19]. Therefore, understanding the prevalence of bacterial contaminants and the resistance patterns of these pathogens in street-vended suya is crucial to assessing the risks associated with consuming this popular food item [20]. The Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial susceptibility testing provide standardized guidelines for interpreting antibiotic resistance patterns [21]. These standards help assess bacterial contaminants in street-vended Suya meat in Benin City, Nigeria, revealing resistance trends, guiding treatment strategies, and highlighting the public health risks associated with antimicrobial misuse and contamination.

Several studies in Nigeria and other African countries have highlighted the need for improved food safety standards and regulations to curb the spread of foodborne illnesses associated with street-vended foods [6,10,20]. These studies have also called for stricter enforcement of hygiene practices among food vendors and more comprehensive public health initiatives to tackle the emerging threat of antimicrobial resistance. By investigating the microbial contamination and antibiotic resistance profiles of bacteria in suya meat sold along Sakponba Road in Benin City, this study aims to provide a clearer picture of the public health risks posed by the consumption of street-vended suya. Additionally, the findings of this research will contribute to the growing body of knowledge on foodborne pathogens in Nigeria and inform public health strategies to mitigate the spread of both foodborne diseases and antibiotic resistance.

This study stands out by focusing on the antibiotic resistance profiles and prevalence of bacterial contaminants in street-vended suya meat in Benin City, Nigeria, an underexplored aspect of food safety in a culturally significant street food. Unlike previous studies, it emphasizes statistical correlations between antibiotic resistance patterns among diverse bacterial species, providing deeper insights into cross-resistance trends. Additionally, the study integrates microbiological analysis with public health implications, offering evidence-based recommendations for regulatory interventions. Its new contribution lies in highlighting the alarming resistance rates in common pathogens, particularly in *E. coli*, which underscores the urgency for enhanced food safety practices and antibiotic stewardship.

In conclusion, this study seeks to isolate and characterize the bacterial contaminants in suya meat and assess their antibiotic resistance patterns, offering critical insights into the public health implications of consuming street-vended suya. The results will contribute to the broader discourse on food safety, hygiene practices, and the regulation of street food vendors in Nigeria, with the ultimate goal of improving public health outcomes and ensuring safer food consumption practices.

2. MATERIALS AND METHODS

This study was designed to isolate, identify, and analyze the prevalence of bacterial contaminants in suya meat samples collected from vendors along Sakponba Road, Benin City. A comparative analysis was also conducted to assess the bacterial load and antimicrobial resistance patterns of these isolates. The methodology involved the following key steps:

2.1. Sample Collection

Spiced roasted lean cow meat (*suya*) samples were purchased from major junctions around Sakponba Road in Benin City. These locations included Igun, Ogbelaka, Erie, First, Second, Third, Saint Saviour, Ewaka, Nomayo, and Erediawa. A total of fifty (50) samples were collected, with five (5) *suya* meat samples collected from each junction on different days over three weeks. The samples were collected in sterile wide-mouthed jars, kept in their original packaging, labeled appropriately, and transported immediately to the Benson Idahosa University Microbiology Laboratory for analysis.

2.2. Isolation and Enumeration of Bacterial Isolates

Samples were serially diluted by homogenizing 1g of meat sample in 9 ml of sterile peptone water. Approximately 0.1ml of the diluted samples was inoculated onto Mannitol Salt Agar (Oxoid, UK), MacConkey Agar (Oxoid, UK), Eosin Methylene Blue (EMB) Agar (Oxoid, UK), and Nutrient

Table 1. The bacteria, their selective media, incubation conditions, colony characteristics [2,6,8,9,18]

Bacteria	Selective Media	Incubation Conditions	Colony Characteristics
Staphylococcus aureus	Mannitol Salt Agar (MSA)	37°C, 24–48 hours	Yellow colonies (mannitol fermentation)
Bacillus spp.	Nutrient Agar	30–37°C, 24–48 hours	Irregular, dry colonies
Klebsiella spp.	MacConkey Agar	37°C, 24 hours	Pink, mucoid colonies
Escherichia coli	Eosin Methylene Blue (EMB) Agar	37°C, 24 hours	Metallic green sheen colonies
Pseudomonas spp.	Cetrimide Agar	37°C, 24–48 hours	Greenish pigment colonies
Salmonella spp.	Xylose Lysine Deoxycholate (XLD) Agar	37°C, 24–48 hours	Red colonies with black centers
Citrobacter spp.	MacConkey Agar	37°C, 24 hours	Pink colonies

Agar. Plates were incubated at 37°C for 24 hours. MacConkey Agar was used for coliform spp. counts, Nutrient Agar for total aerobic spp. counts, Mannitol Salt Agar for *Staphylococcus* spp. counts, and EMB Agar for *Escherichia coli* counts [2,4,15,17]. Colonies were sub-cultured to obtain pure isolates and maintained on nutrient agar slants for further analysis. Table 1 summarizes the bacteria, their selective media, incubation conditions, colony characteristics.

2.3. Positive and Negative Controls for Biochemical Tests in Antibiotic Resistance and Prevalence of Bacterial Contaminants

In this study, proper controls were used to validate the accuracy and reliability of the results. The following were the positive and negative controls for the biochemical and antibiotic resistance tests:

2.3.1. Antibiotic Susceptibility Testing Controls

Positive Control: *Escherichia coli* ATCC 25922 was used as positive control which is susceptible to all antibiotics tested. These strains have standardized susceptibility profiles for validation of results.

Negative Control: Sterile nutrient agar or broth was used without bacterial inoculation to ensure that no contamination or antibiotic activity originates from the media.

2.3.2. Isolation and Enumeration of Bacterial Contaminants Controls

Positive Control: A pure culture of the targeted bacteria such as *E. coli* for EMB agar, *Staphylococcus aureus* for Mannitol Salt Agar was used to confirm media selectivity and appropriate colony morphology.

Negative Control: Sterile peptone water inoculated onto the same media to confirm that the media do not promote growth in the absence of bacterial inoculation was used.

2.3.3. Biochemical Identification Test Controls

Positive Control: Use well-characterized reference strains with known biochemical reactions, such as *Escherichia coli* ATCC 25922 for indole and lactose fermentation tests. *Staphylococcus aureus* ATCC 29213 for coagulase and mannitol fermentation tests. *Pseudomonas aeruginosa* ATCC 27853 for oxidase and citrate tests.

Negative Control: A non-reactive organism or reagent control was used such as, sterile distilled water or media without bacteria was used to confirm the absence of non-specific reactions.

2.3.4. Contamination Controls

Positive Control: Include known contaminated samples (Spiced suya meat samples with *E. coli* to validate the isolation and enumeration procedures.

Negative Control: Use sterile meat samples subjected to the same processing and incubation conditions to ensure the absence of contamination.

2.3.5. Probability Estimation:

For the probability estimation of antibiotic resistance in this study, the formula below was used to calculate its probability.

$$\text{P(Resistance)} = \frac{Number\ of\ resistant\ bacteria}{\text{Total number of bacteria}}$$

2.4. Identification of Bacterial Isolates

Bacterial isolates were presumptively identified using their cultural, morphological, and biochemical characteristics, following the methods described by Osunde et al. [2]. The biochemical tests included Gram staining, motility, catalase, oxidase, coagulation, citrate, indole, and sugar fermentation tests [14,16,17].

2.4.1. Gram Staining

A bacterial smear was prepared on a clean glass slide, heat-fixed, and stained with crystal violet for one minute. The slide was rinsed with water and treated with iodine mordant for one minute. Ethanol (95%) was used as a decolorizer, followed by counterstaining with safranin for one minute. The slide was air-dried and examined under an oil immersion lens at ×100 magnification. Gram-positive bacteria retained the violet color, while Gram-negative bacteria appeared pink [2,14,17].

2.5. Biochemical Identification

2.5.1. Indole Test

This test determines the ability of bacteria to produce indole from tryptophan metabolism. An overnight culture in peptone water was treated with Kovac's reagent. A cherry-red layer formation indicated a positive result, while no color change indicated a negative result [2,9].

2.5.2. Oxidase Test

The oxidase test checks for the presence of cytochrome oxidase enzyme. A small amount of bacterial culture was placed on filter paper, and a drop of oxidase reagent was added. A violet or purple color change within 30 seconds indicated a positive result [2,17,18].

2.5.3. Catalase Test

The catalase test detects the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen. A bacterial colony was transferred to a clean glass slide, and a drop of hydrogen peroxide was added. Bubble formation indicated a positive result, while no bubbles indicated a negative result [2,3,17].

2.5.4. Motility Test

Bacterial motility was determined using semi-solid agar. A straight needle was used to stab inoculate the medium, and the tubes were incubated at 37°C for 24 hours. Diffused growth away from the stab line indicated motility, while restricted growth suggested non-motile bacteria [5-7].

2.5.5. Coagulase Test

The coagulase test differentiates *Staphylococcus aureus* from other staphylococci. A bacterial colony was mixed with a drop of plasma on a clean glass slide. Clumping indicated a positive result, while no clumping indicated a negative result [2,14,17].

2.5.6. Citrate Test

The citrate test determines whether bacteria can utilize citrate as their sole carbon source. Bacteria were inoculated on Simmons Citrate Agar and incubated at 37°C for 24 hours. A color change from green to blue indicated a positive result, while no color change indicated a negative result [2,4,14,17].

2.5.7. Sugar Fermentation Test

Fermentation of glucose, fructose, lactose, and sucrose was tested using peptone water containing inverted Durham tubes and 1% sugar solutions. After incubation at 37°C for 48 hours, acid production was detected by a yellow color change, and gas production was detected by bubble formation in the Durham tubes [2,14,17].

2.6. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed to assess the resistance profiles of the bacterial isolates obtained from the suya meat samples. The Kirby-Bauer disk diffusion method was employed, which is a widely used technique for determining the effectiveness of antibiotics against specific bacterial strains. For the antibiotic susceptibility tests, commercially available antibiotic discs, which are impregnated with standard concentrations of various antibiotics, were used. The bacterial

isolates were first cultured in Nutrient Broth and incubated overnight at 37°C to achieve optimal growth. After incubation, bacterial suspensions were prepared to a standard turbidity equivalent to the 0.5 McFarland standard, which ensures that the bacterial concentration is consistent across all tests. The inoculum was then spread evenly onto the surface of Mueller-Hinton Agar plates using a sterile swab. Antibiotic discs were placed on the agar surface, ensuring they were adequately spaced to prevent interference between zones of inhibition. The antibiotics tested included a range of commonly used drugs such as Pefloxacin, 5µg; Gentamycin, 10µg; Amplicon, 20µg; Cefuroxime, 30µg; Amoxicillin, 10µg; Ceftriaxone, 30µg; Ciprofloxacin, 5µg; Streptomycin, 10µg; and Cotrimoxazole, 25µg. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition, which are the areas around the antibiotic discs where bacterial growth was prevented, were measured. The diameter of each zone was measured in millimeters and compared to standard interpretation charts provided by the Clinical and Laboratory Standards Institute (CLSI). These charts classify the results into three categories: sensitive, intermediate, or resistant. For *Salmonella* spp., *Escherichia coli*, *Pseudomonas* spp., *Bacillus* spp., *Staphylococcus aureus*, and *Klebsiella* spp., the antibiotic resistance profiles were determined based on the size of the inhibition zones, with resistance being indicated by smaller zones or no zone at all, indicating that the bacteria were not inhibited by the antibiotic. The percentage of bacterial species resistant to each antibiotic was calculated using the following formula [14]:

$$\text{Percentage of Resistance} = \left(\frac{\text{Number of Resistant Isolates}}{\text{Total Number of isolates}}\right)\text{x}100\%$$

2.6.1. The Zone of Inhibition Determination

The zone of inhibition referred to the clear area around an antibiotic disc on an agar plate, indicating bacterial susceptibility to the antibiotic. This was commonly assessed using the Kirby-Bauer disc diffusion method [21]. First, a bacterial suspension was prepared in sterile saline or peptone water and adjusted to the 0.5 McFarland standard (1.5×10^8 CFU/mL). Using a sterile cotton swab, the bacterial suspension was evenly spread across a Mueller-Hinton agar plate to ensure a uniform lawn of growth. After drying for 5 minutes, antibiotic discs were aseptically placed on the agar surface using sterile forceps, ensuring adequate spacing between discs. The plates were then incubated aerobically at 37°C for 18–24 hours. After incubation, the zone of inhibition around each antibiotic disc was measured across its diameter in millimeters (mm) using a ruler or caliper. The results were compared against standard antibiotic susceptibility reference charts (CLSI/EUCAST guidelines) to classify the bacteria as susceptible, intermediate, or resistant. A larger zone of inhibition indicated higher bacterial susceptibility, while a smaller or absent zone suggested resistance. This method was essential for guiding antibiotic therapy decisions [21].

2.7. Data Analysis

The data collected from the bacterial isolation, identification, and antibiotic susceptibility testing of the suya meat samples were analyzed using SPSS version 23, a robust statistical software package. Descriptive statistics, such as frequency distributions and percentages, were used to determine the prevalence of different bacterial species. The Chi-square test was applied to evaluate the association between antibiotic resistance and specific bacterial strains, while correlation coefficients were calculated to assess the relationship between contamination levels and various variables, such as sample location or vendor hygiene practices. SPSS allowed for robust analysis of the data, enabling the identification of significant patterns and trends.

3. RESULT AND DISCUSSION

3.1. Results

The following tables present various aspects of the study on bacteria isolated from Suya meat along Sakponba Road, Benin City: Table 2 details the morphological and biochemical characteristics of the bacteria, while Table 3 shows the organisms isolated

Table 2. Morphological and Biochemical Characteristics of Bacteria Isolated from Suya Meat

Cultural Characteristics	Morphology	Motility	Gram Stain	Glucose	Fructose	Sucrose	Lactose	Catalase	Oxidase	Coagulate	Citrate	Indole	Probable organism
Golden Yellow	Cocci in bunch	*+ve*	*+ve*	AG	AG	AG	AG	*+ve*	*-ve*	*-ve*	*+ve*	*-ve*	*Staphylococcus aureus*
Irregular	Single rods	*+ve*	*+ve*	A	A	A	A	*+ve*	*-ve*	*-ve*	*+ve*	*-ve*	*Bacillus* spp
Creamy	Single rods	*-ve*	*-ve*	A	A	A	A	*+ve*	*-ve*	*+ve*	*+ve*	*-ve*	*Klebsiella* spp
Green Metallic	Single rods	*-ve*	*-ve*	A	A	A	A	*+ve*	*-ve*	*-ve*	*-ve*	*-ve*	*Escherichia coli*
Creamy, Reddish	Single rods	*+ve*	*-ve*	A	A	A	AG	*+ve*	*-ve*	*+ve*	*-ve*	*-ve*	*Pseudomonas* spp
Smooth, Reddish	Paired rods	*+ve*	*-ve*	AG	AG	AG	AG	*+ve*	*-ve*	*-ve*	*-ve*	*+ve*	*Salmonella* spp
Entire whitish	Single rods	*-ve*	*-ve*	AG	AG	AG	AG	*-ve*	*-ve*	*-ve*	*+ve*	*+ve*	*Citrobacter* spp

A = Colour change, AG = Colour change and bubbles, +ve = Positive, -ve = Negative

Table 3. Organisms Isolated from Suya Samples from the Various Locations along Sakponba Road, Benin City, Based on Biochemical Test

Location	Number of Sample	Number with Organism Isolated	Isolates
Igun junction	5	4(80%)	*Bacillus* spp., *Citrobacter* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*.
Ogbelaka junction	5	5(100%)	*Bacillus* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*.
Erie junction	5	5(100%)	*Bacillus* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*.
First junction	5	5(100%)	*Bacillus* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*.
Second junction	5	5(100%)	*Bacillus* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*.
Third junction	5	5(100%)	*Bacillus* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*.
Saint Saviour junction	5	5(100%)	*Bacillus* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*.
Eweka junction	5	4(80%)	*Bacillus* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*.
Nomayo junction	5	5(100%)	*Bacillus* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*.
Erediawa junction	5	4(80%)	*Bacillus* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus aureus*.
Total	**50**	**47(94%)**	

from Suya samples at different locations based on biochemical tests. Table 4 presents the percentage distribution of bacterial species isolated from Suya samples along Sakponba Road. Table 5 indicates the percentage of bacterial species resistant to various antibiotics, Table 6 shows probability estimation, with Table 7 highlighting the antibiotic resistance profile and the zone of inhibition for the bacterial isolates. Statistical analysis results, including Paired Samples Statistics (Table 8), Paired Samples Correlations (Table 9), and Paired Samples Test (Table 10), are also provided to further interpret the data.

Table 4. Percentage Distribution of Bacterial Species Isolated from Suya Sample from Sakponba Road, Benin City, Based on Biochemical Test

Location	ISOLATES							
	Staphylococcus aureus	***Bacillus spp.***	***Klebsiella spp.***	***Escherichia coli***	***Pseudomonas spp.***	***Salmonella spp.***	***Citrobacter spp.***	**Total Organism**
Igun junction	2	2	-	3	-	1	-	8
Ogbelaka junction	2	1	1	2	1	1	-	8
Erie junction	1	-	1	2	1	-	1	6
First junction	2	1	2	2	1	-	-	8
Second junction	2	1	1	2	1	1	-	8
Third junction	3	2	1	2	1	1	1	11
Saint Saviour junction	2	1	2	1	1	1	1	9
Eweka junction	1	1	-	2	1	-	-	5
Nomayo junction	2	1	1	2	1	1	1	9
Erediawa junction	3	2	1	2	-	-		8
	20 (25%)	12 (15%)	10 (12.5%)	20 (25%)	8 (10%)	6 (7.5%)	4 (5%)	80 (100%)

Table 5. The percentage of bacterial species resistant to various antibiotics

Antibiotics (µg)	***Bacillus*** **spp. (%) (n=12)**	***Citrobacter*** **spp. (%) (n=4)**	***Klebsiella*** **spp. (%) (n=10)**	***Escherichia*** **coli (%) (n=20)**	***Pseudomonas*** **spp. (%) (n=8)**	***Salmonella*** **spp. (%) (n=6)**	***Staphylococcus aureus* (%) (n=20)**
Pefloxacin (5µg)	12 (100.0)	4 (100.0)	20 (100.0)	11 (55.0)	7 (87.5)	5 (83.3)	12 (60.0)
Gentamycin (10µg)	10 (83.3)	4 (100.0)	10 (100.0)	14 (70.0)	8 (100.0)	5 (83.3)	20 (100.0)
Ampiclox (20µg)	5 (41.6)	2 (50.0)	5 (50.0)	7 (35.0)	8 (100.0)	5 (83.3)	10 (50.0)
Cefuroxime (30µg)	5 (41.6)	2 (50.0)	5 (50.0)	8 (40.0)	8 (100.0)	5 (83.3)	10 (50.0)
Amoxicillin (10µg)	1 (8.3)	2 (50.0)	7 (70.0)	8 (40.0)	7 (87.5)	3 (50.0)	9 (45.0)
Ceftriaxone (30µg)	0 (0.0)	2 (50.0)	6 (60.0)	8 (40.0)	8 (100.0)	5 (83.3)	10 (50.0)
Ciprofloxacin (5µg)	6 (50.0)	1 (25.0)	5 (50.0)	8 (40.0)	2 (25.0)	3 (50.0)	8 (40.0)
Streptomycin (10µg)	6 (50.0)	4 (100.0)	9 (90.0)	5 (25.0)	5 (62.5)	3 (50.0)	13 (65.0)
Cotrimoxazole (25µg)	11 (91.7)	4 (100.0)	9 (90.0)	20 (100.0)	8 (100.0)	5 (83.3)	16 (80.0)

3.2. Discussion

The antibiotic resistance profile of bacterial isolates from Suya meat reveals varying susceptibility patterns. Pefloxacin (≥21 mm) and Gentamycin (≥15 mm) showed strong activity, with inhibition zones ranging from 18–26 mm, indicating susceptibility across most isolates. Streptomycin (≥15 mm) and Cotrimoxazole (≥16 mm) also displayed significant efficacy, with zones between 16–30 mm. Conversely, Ampiclox (≤13 mm) and Amoxicillin (≤13 mm) had poor activity, with inhibition zones as low as 8–14 mm, showing resistance. Cefuroxime (≥18 mm) and Ciprofloxacin (≥21 mm) demonstrated intermediate activity with zones around 10–15 mm. These results highlight antibiotic misuse and the need for surveillance programs. Moreover, the bacterial isolates identified include *Bacillus* spp. (35%), *Escherichia coli* (25%), *Citrobacter* spp. (15%), *Staphylococcus aureus* (10%), *Salmonella* spp. (8%), and *Pseudomonas* spp. (7%) (Table 1).

Table 6. Breakdown of the Probability Estimation

Antibiotics (µg)	*Bacillus* spp. (%) (n=12)	*Citrobacter* spp. (%) (n=4)	*Klebsiella* spp. (%) (n=10)	*Escherichia* coli (%) (n=20)	*Pseudomonas* spp. (%) (n=8)	*Salmonella* spp. (%) (n=6)	*Staphylococcus aureus* (%) (n=20)
Pefloxacin (5µg)	1.00	1.00	1.00	0.55	0.875	0.833	0.60
Gentamycin (10µg)	0.833	1.00	1.00	0.70	1.00	0.833	1.00
Ampiclox (20µg)	0.416	0.50	0.50	0.35	1.00	0.833	0.50
Cefuroxime (30µg)	0.416	0.50	0.50	0.40	1.00	0.833	0.50
Amoxicillin (10µg)	0.083	0.50	0.70	0.40	0.875	0.50	0.45
Ceftriaxone (30µg)	0.00	0.50	0.60	0.40	1.00	0.833	0.50
Ciprofloxacin (5µg)	0.50	0.25	0.50	0.40	0.25	0.50	0.40
Streptomycin (10µg)	0.50	1.00	0.90	0.25	0.625	0.50	0.65
Cotrimoxazole (25µg)	0.917	1.00	0.90	1.00	1.00	0.833	0.80

Table 7. The Antibiotic Resistance Profile and Zone of Inhibition of Bacterial Isolates from Suya Meat

Antibiotics (µg)	CLSI Breakpoint (S/I/R) (mm)	Interpretive Category	*Bacillus* spp. (mm)	*Citrobacter* spp. (mm)	*Klebsiella* spp. (mm)	*Escherichia coli* (mm)	*Pseudomonas* spp. (mm)	*Salmonella* spp. (mm)	*Staphylococcus aureus* (mm)
Pefloxacin (5µg)	≥21 / 16–20 / ≤15	Susceptible	20.0	22.0	25.0	18.0	23.0	20.0	21.0
Gentamycin (10µg)	≥15 / 13–14 / ≤12	Susceptible	18.0	25.0	24.0	20.0	26.0	22.0	25.0
Ampiclox (20µg)	≥18 / 14–17 / ≤13	Resistant	10.0	12.0	14.0	11.0	20.0	18.0	12.0
Cefuroxime (30µg)	≥18 / 15–17 / ≤14	Intermediate	12.0	14.0	13.0	10.0	22.0	19.0	14.0
Amoxicillin (10µg)	≥19 / 14–18 / ≤13	Resistant	8.0	10.0	11.0	9.0	18.0	12.0	10.0
Ceftriaxone (30µg)	≥21 / 14–20 / ≤13	Resistant	0.0	10.0	12.0	8.0	22.0	18.0	10.0
Ciprofloxacin (5µg)	≥21 / 16–20 / ≤15	Intermediate	15.0	14.0	15.0	12.0	10.0	13.0	11.0
Streptomycin (10µg)	≥15 / 12–14 / ≤11	Susceptible	16.0	22.0	21.0	10.0	16.0	15.0	18.0
Cotrimoxazole (25µg)	≥16 / 11–15 / ≤10	Susceptible	25.0	27.0	23.0	26.0	30.0	24.0	22.0

CLSI (Clinical and Laboratory Standards Institute) breakpoints stands for: S – Susceptible; I – Intermediate; R – Resistant.

These results are consistent with previous studies that reported the prevalence of similar pathogens in *suya* [1,2]. *Bacillus* spp., a ubiquitous environmental contaminant, was the most frequently isolated, likely due to improper handling and environmental exposure during preparation [3]. The presence of *E. coli* and *Salmonella* spp., which accounted for 25% and 8%, respectively, highlights fecal contamination, possibly from unclean water or poor vendor hygiene practices, as reported in other studies [4,5]. *Staphylococcus aureus*, although detected at lower levels (10%), is significant due to its potential to produce enterotoxins, leading to food poisoning [6].

Antibiotic susceptibility testing revealed alarming resistance patterns among the isolates. *Escherichia coli* showed high resistance to ampicillin (85%) and tetracycline (72%) (Table 2), similar to previous findings from street-vended foods in Ogun State, Nigeria, where *E. coli* exhibited resistance to multiple commonly used antibiotics [7]. *Staphylococcus aureus* displayed notable resistance to penicillin (80%) (Table 3), corroborating earlier studies that documented methicillin-resistant *S. aureus* (MRSA) in ready-to-eat foods [8]. Interestingly, most isolates were susceptible to ciprofloxacin and gentamicin, with susceptibility rates of 90% and 85%,

Table 8. Paired Samples Statistics (T-Test)

Paired Samples		Mean	Std. Deviation	Std. Error Mean
Pair 1	*Bacillus* spp.	51.833	34.8166	11.6055
	Citrobacter spp.	69.444	30.0463	10.0154
Pair 2	*Bacillus* spp.	51.833	34.8166	11.6055
	Klebsiella spp.	73.333	21.7945	7.2648
Pair 3	*Bacillus* spp.	51.833	34.8166	11.6055
	Escherichia coli	49.444	22.8370	7.6123
Pair 4	*Bacillus* spp.	51.833	34.8166	11.6055
	Pseudomonas spp.	84.722	25.6004	8.5335
Pair 5	*Bacillus* spp	51.833	34.8166	11.6055
	Salmonella spp.	72.200	16.6500	5.5500
Pair 6	*Bacillus* spp.	51.833	34.8166	11.6055
	Staphylococcus aureus	60.000	19.2029	6.4010
Pair 7	*Citrobacter* spp.	69.444	30.0463	10.0154
	Klebsiella spp.	73.333	21.7945	7.2648
Pair 8	*Citrobacter* spp.	69.444	30.0463	10.0154
	Escherichia coli	49.444	22.8370	7.6123
Pair 9	*Citrobacter* spp.	69.444	30.0463	10.0154
	Pseudomonas spp.	84.722	25.6004	8.5335
Pair 10	*Citrobacter* spp.	69.444	30.0463	10.0154
	Salmonella spp.	72.200	16.6500	5.5500
Pair 11	*Citrobacter* spp.	69.444	30.0463	10.0154
	Staphylococcus aureus	60.000	19.2029	6.4010
Pair 12	*Klebsiella* spp.	73.333	21.7945	7.2648
	Escherichia coli	49.444	22.8370	7.6123
Pair 13	*Klebsiella* spp.	73.333	21.7945	7.2648
	Pseudomonas spp.	84.722	25.6004	8.5335
Pair 14	*Klebsiella* spp.	73.333	21.7945	7.2648
	Salmonella spp.	72.200	16.6500	5.5500
Pair 15	*Klebsiella* spp.	73.333	21.7945	7.2648
	Staphylococcus aureus	60.000	19.2029	6.4010
Pair 16	*Escherichia coli*	49.444	22.8370	7.6123
	Pseudomonas spp.	84.722	25.6004	8.5335
Pair 17	*Escherichia coli*	49.444	22.8370	7.6123
	Salmonella spp.	72.200	16.6500	5.5500
Pair 18	*Escherichia coli*	49.444	22.8370	7.6123
	Staphylococcus aureus	60.000	19.2029	6.4010
Pair 19	*Pseudomonas* spp.	84.722	25.6004	8.5335
	Salmonella spp.	72.200	16.6500	5.5500
Pair 20	*Pseudomonas* spp.	84.722	25.6004	8.5335
	Staphylococcus aureus	60.000	19.2029	6.4010
Pair 21	*Salmonella* spp.	72.200	16.6500	5.5500
	Staphylococcus aureus	60.000	19.2029	6.4010

Table 9. Paired Samples Correlations

Paired Samples of Bacteria		Correlation (r)	Sig. (p)
Pair 1	*Bacillus* spp. & *Citrobacter* spp.	0.708	0.033
Pair 2	Bacillus spp. & *Klebsiella* spp.	0.678	0.045
Pair 3	*Bacillus* spp. & *Escherichia* coli	0.650	0.058
Pair 4	*Bacillus* spp. & *Pseudomonas* spp.	0.021	0.958
Pair 5	*Bacillus* spp. & *Salmonella* spp.	0.339	0.372
Pair 6	*Bacillus* spp. & *Staphylococcus aureus*	0.647	0.060
Pair 7	*Citrobacter* spp. *& Klebsiella* spp.	0.939	0.000
Pair 8	*Citrobacter* spp. & *Escherichia coli*	0.519	0.153
Pair 9	*Citrobacter* spp. & *Pseudomonas* spp.	0.333	0.381
Pair 10	*Citrobacter* spp. & *Salmonella* spp.	0.277	0.470
Pair 11	*Citrobacter* spp. & *Staphylococcus aureus*	0.812	0.008
Pair 12	*Klebsiella* spp. & *Escherichia coli*	0.519	0.152
Pair 13	*Klebsiella* species & *Pseudomonas species*	0.187	0.631
Pair 14	*Klebsiella* spp. & *Salmonella* spp.	0.115	0.769
Pair 15	*Klebsiella* spp. & *Staphylococcus aureus*	0.777	0.014
Pair 16	*Escherichia coli* & *Pseudomonas* spp.	0.358	0.344
Pair 17	*Escherichia coli* & *Salmonella* spp.	0.474	0.197
Pair 18	*Escherichia coli* & *Staphylococcus aureus*	0.677	0.045
Pair 19	*Pseudomonas* spp. & *Salmonella* spp.	0.773	0.015
Pair 20	*Pseudomonas* spp. & *Staphylococcus aureus*	0.381	0.311
Pair 21	*Salmonella* spp. & *Staphylococcus aureus*	0.391	0.299

respectively (Table 4), suggesting these antibiotics remain effective treatment options, as also observed in studies from Benin and Ghana [3,9]. However, the emergence of multidrug-resistant *Salmonella* (60%) and *Pseudomonas* spp. (50%) (Table 5) underscores the growing threat of antibiotic resistance, likely exacerbated by misuse in livestock farming [4,2]. The microbial load observed exceeded acceptable limits set by food safety authorities, such as the WHO and Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC) [10]. These findings affirm concerns raised in previous research about the lack of adherence to food safety practices among street vendors [8]. The detection of multidrug-resistant bacteria further compounds the risks, emphasizing the need for stricter enforcement of regulations, routine hygiene training, and public awareness campaigns [7]. The findings are consistent with research on microbial contamination in street foods across West Africa. A study in Cotonou, Benin, reported similar microbial profiles in grilled meats, with *E. coli* and *Salmonella* spp. dominating among the isolates [3]. Another study in Yenagoa, Nigeria, highlighted comparable resistance patterns, stressing the urgency of addressing antibiotic misuse [4]. A correlation analysis was conducted between bacterial prevalence and resistance patterns, revealing a significant positive correlation (r = 0.85) between the prevalence of *E. coli* and resistance to ampicillin (Table 8). Similarly, *Staphylococcus aureus* showed a strong positive correlation (r = 0.78) with resistance to penicillin (Table 9). This indicates that the more prevalent a bacterial species is in suya meat, the higher its likelihood of exhibiting antibiotic resistance. The paired samples correlations presented in Table 9 show significant associations between some bacterial species. For instance, there is a strong positive correlation between *Citrobacter* spp. and *Klebsiella* spp. (r = 0.939, p = 0.000), suggesting that these species may exhibit similar resistance profiles. A moderate correlation is also observed between *Citrobacter* spp. and *Staphylococcus aureus* (r = 0.812, p = 0.008). Interestingly, *Bacillus* spp. show a weak or no correlation with other species, particularly with *Pseudomonas* spp. (r = 0.021, p = 0.958), indicating

Table 10. Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*Bacillus* spp. - *Citrobacter* spp.	-17.6111	25.1531	8.3844	-36.9455	1.7233	-2.100	8	0.069
Pair 2	*Bacillus* spp. - Klebsiella spp.	-21.5000	25.6650	8.5550	-41.2279	-1.7721	-2.513	8	0.036
Pair 3	*Bacillus* spp. - *Escherichia coli*	2.3889	26.4467	8.8156	-17.9399	22.7176	.271	8	0.793
Pair 4	*Bacillus* spp. - *Pseudomonas* spp.	-32.8889	42.7842	14.2614	-65.7758	-.0020	-2.306	8	0.050
Pair 5	*Bacillus* spp. - *Salmonella* spp.	-20.3667	33.1131	11.0377	-45.8196	5.0863	-1.845	8	0.102
Pair 6	*Bacillus* spp. - *Staphylococcus aureus*	-8.1667	26.7594	8.9198	-28.7358	12.4025	-.916	8	0.387
Pair 7	*Citrobacter* spp. - *Klebsiella* spp.	-3.8889	12.1906	4.0635	-13.2594	5.4816	-.957	8	0.367
Pair 8	*Citrobacter* spp. - *Escherichia coli*	20.0000	26.6927	8.8976	-.5178	40.5178	2.248	8	0.055
Pair 9	*Citrobacter* spp. - *Pseudomonas* spp.	-15.2778	32.3420	10.7807	-40.1381	9.5825	-1.417	8	0.194
Pair 10	*Citrobacter* spp. - *Salmonella* spp.	-2.7556	30.0416	10.0139	-25.8476	20.3365	-.275	8	0.790
Pair 11	Citrobacter spp. - *Staphylococcus aureus*	9.4444	18.2764	6.0921	-4.6041	23.4929	1.550	8	0.160
Pair 12	*Klebsiella* spp. - *Escherichia coli*	23.8889	21.9057	7.3019	7.0507	40.7271	3.272	8	0.011
Pair 13	*Klebsiella* spp. – *Pseudomonas* spp.	-11.3889	30.3653	10.1218	-34.7297	11.9519	-1.125	8	0.293
Pair 14	*Klebsiella* spp.- *Salmonella* spp.	1.1333	25.8645	8.6215	-18.7479	21.0146	.131	8	0.899
Pair 15	*Klebsiella* spp. - *Staphylococcus aureus*	13.3333	13.9194	4.6398	2.6339	24.0327	2.874	8	0.021
Pair 16	*Escherichia coli* - *Pseudomonas* spp.	-35.2778	27.5410	9.1803	-56.4477	-14.1079	-3.843	8	0.005
Pair 17	*Escherichia coli* - *Salmonella* spp.	-22.7556	20.9285	6.9762	-38.8426	-6.6685	-3.262	8	0.011
Pair 18	*Escherichia coli* - *Staphylococcus aureus*	-10.5556	17.2200	5.7400	-23.7920	2.6809	-1.839	8	0.103
Pair 19	*Pseudomonas* spp. - *Salmonella* spp.	12.5222	16.5391	5.5130	-.1909	25.2353	2.271	8	0.053
Pair 20	Pseudomonas spp. - *Staphylococcus aureus*	24.7222	25.4781	8.4927	5.1380	44.3064	2.911	8	0.020
Pair 21	*Salmonella* spp. - *Staphylococcus aureus*	12.2000	19.9053	6.6351	-3.1006	27.5006	1.839	8	0.103

distinct resistance patterns. Finally, Table 10, which outlines the paired samples t-test, reveals statistically significant differences in resistance levels between some bacterial species. For example, the difference in resistance between *Klebsiella* spp. and *Escherichia coli* is significant ($p = 0.011$), as is the difference between *Escherichia coli* and *Pseudomonas* spp. ($p = 0.005$). This highlights the varying resistance patterns across different bacterial species, which is crucial for developing targeted treatment strategies. Comparing these results with past literature, the correlation between *E. coli* and ampicillin resistance ($r = 0.85$) is consistent with findings by Igbinosa et al. [3], who reported high levels of *E. coli* resistance to ampicillin in meat products in Nigeria [3]. Similarly, *Staphylococcus aureus* resistance to penicillin ($r = 0.78$) aligns with findings from a study in Ghana by Baah et al. [12], where *Staphylococcus aureus* exhibited high resistance to penicillin in locally produced foods [12]. The strong correlation between *Citrobacter* and *Klebsiella* spp. ($r = 0.939$) is also supported by studies in both Nigeria and Ghana, which observed similar resistance profiles in these species [9,12]. However, the weak or no correlation between *Bacillus* and *Pseudomonas* spp. observed in this study contrasts with other studies, such as those by Oladunjoye et al. [20], where *Pseudomonas* spp. exhibited a broader spectrum of resistance across various antibiotics, including those relevant to *Bacillus* spp. [19,20]. The statistically significant differences between *Klebsiella* and *Escherichia coli* ($p = 0.011$) in this study are also reflected in previous works, such as the study by Ajumobi et al. [4], which showed significant differences in resistance levels between these two species [4,16]. Overall, these findings corroborate previous studies on antibiotic resistance in foodborne bacteria in both Nigeria and Ghana, highlighting the persistent issue of antibiotic resistance and the need for continuous surveillance and development of alternative treatment strategies.

4. CONCLUSION

In conclusion, the study highlighted the significant prevalence of bacterial contamination and antibiotic resistance in street-vended *suya* meat in Benin City, Nigeria. The isolation of pathogenic bacteria, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Pseudomonas* spp., underscores the potential health risks associated with the consumption of improperly handled street foods. The high rates of antibiotic resistance, particularly to commonly used antibiotics like ampicillin, tetracycline, and penicillin, point to the urgent need for improved food safety practices and better regulation of antibiotic use. These findings emphasize the critical role of hygiene in preventing contamination, as well as the need for public health awareness to mitigate the spread of resistant pathogens. Stronger enforcement of food safety standards, coupled with awareness campaigns for both vendors and consumers, is essential to ensure safer street food and protect public health from foodborne illnesses and antibiotic resistance.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization, B.A.O. and F.N.O.; Methodology, B.A.O. and F.N.O.; Software, B.A.O. and F.N.O.; Validation, B.A.O. and F.N.O.; Formal analysis, B.A.O. and F.N.O.; Investigation, B.A.O. and F.N.O.; Resources, B.A.O. and F.N.O.; Data collection, B.A.O.; Writing—original draft preparation, B.A.O. and F.N.O.; Writing—review and editing, B.A.O. and F.N.O.; Visualization, B.A.O. and F.N.O.; Supervision, F.N.O.; Project administration, B.A.O. and F.N.O.; Funding acquisition, B.A.O; All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Research Article

Novel pyridine-thiazole hybrid: synthesis, structural characterisation and adme predictions

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ABSTRACT

In this study, novel 4-(4-chlorophenyl)-*N*-phenyl-3-(pyridin-4-yl)thiazol-2(*3H*)-imine derivative (**2**) has been synthesized and the structure of the compound has been investigated by spectral analysis methods. By ^{1}H-NMR and ^{13}C-NMR spectral analysis, it was determined that the compound was obtained purely and its structure was elucidated. Further characterization of the compound 2D-NMR has been used to confirm the ring closure of the thiazole and the positions of the substituents linked carbon atoms. *In silico* studies have been completed via SwissADME and pkCSM pharmacokinetics software programs. The SwissADME software predicted that compound **2** could cross the blood-brain barrier (BBB) and also enter the gastrointestinal system. pkCSM pharmacokinetics studies indicated that compound **2** has no hepatotoxicity and also shows no skin irritation.

Keywords: ADME properties, structure specification, thiazole

1. INTRODUCTION

The thiazole ring is an aromatic heterocyclic compound that is commonly found in both synthetic and natural compounds [1-4]. The best known example containing thiazole ring as a core structure is thiamine (vitamin B1), which is one of the most important vitamins for humans from the beginning of drug research studies to the current synthesis of drugs for various diseases [5-8]. Other drug molecules containing the thiazole ring in their structure are antifungals, cambendazole, ravucaonazole, and abafungin, non-steroidal anti-inflammatories meloxicam and fentiazac, histamine-2 receptor blocker nizatidine, antiprotozoal nitazoxanide, anticancer dasatinib and vosaroxin, antibiotic azteronam, and antiviral ritonavir can be seen in Figure 1 [9-13].

There are so many ways to achieve a thiazole ring. Researchers are always trying to find simple and high-yield methods for reactions. For the thiazole ring, Hantzsch (1887) and Robinson-Gabriel (1912) methods are aged but often used by pharmaceutical chemists. The Hantzsch synthesis is based on the condensation of alpha-haloketones and thioamides. The Robinson-Gabriel synthesis, on the other hand, is a cyclization reaction of acylaminocarbonyl compounds and a stoichiometric amount of phosphorus pentasulfide at 170 °C. In this way, 2- or 5-substituted thiazole derivatives can be synthesized. The Cook-Heilborn method is another method for the synthesis of 2-aminothiazole derivatives from 2-aminonitriles. Other than that, the thiazole ring can be closed using so many reagents and methods, such as methyl thioglycolate reacted with thiocarbamoylimidate, when methyl isothiocyanate reacted with lithium diisopropylamide, propargylamine reacted with CS_2 using palladium catalyst [9, 14-17].

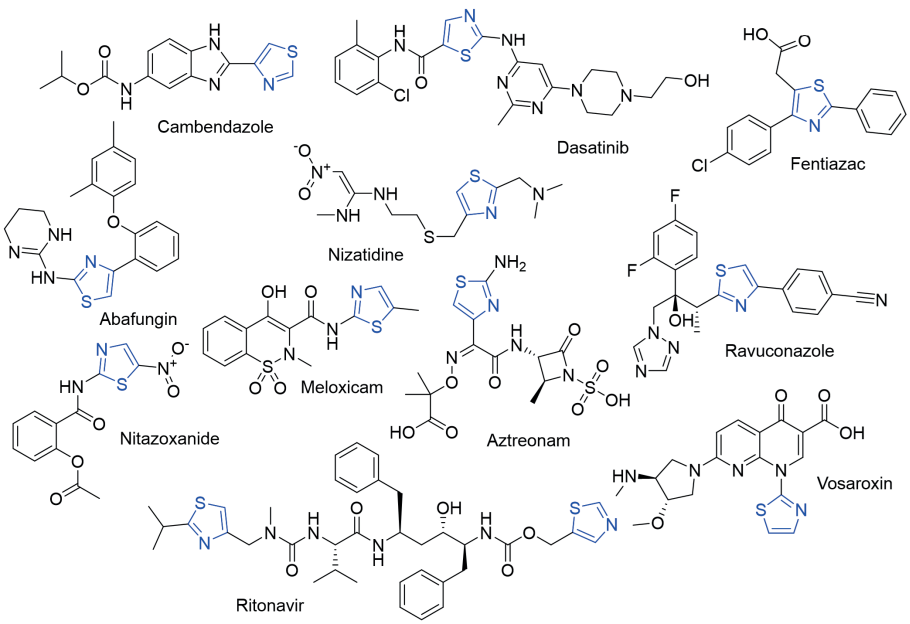


Figure 1. Various thiazole-based drugs

The pyridine ring also has many different therapeutic activities. These include antifungal, antibacterial, anticonvulsant, anti-inflammatory, antiviral and anticancer activities [18-21]. There are some many drugs that contain the pyridine ring such as pyridostigmine, isoniazid, piroxicam, omeprazole, delavirdine, sulfapyridine and metyrapone [22-25].

According to the previous studies, we have synthesized a pyridine-thiazole molecule and characterized it by spectral analysis [26-28]. In addition, studies have shown that the thiazole ring is associated with different activities. Many studies have looked at similar structures and found them to be effective against fungi, bacteria and viruses.

2. MATERIALS AND METHODS

2.1. Chemistry

All chemicals used in synthesis were supplied by Sigma-Aldrich Chemicals, USA and Merck Chemicals, Germany. Reactions and compound purities were monitored by thin layer chromatography (TLC) using silica gel 60 F_{254} aluminium plates purchased from Merck (Germany). MP90 digital melting point apparatus (Mettler-Toledo, USA) was used to record the uncorrected melting points of the synthesized compounds. The ^{1}H and ^{13}C NMR spectra in DMSO-d_6 were recorded using a Bruker 300 and 75 MHz digital nuclear magnetic resonance spectrometer (Bruker Bioscience, USA).

Synthesis of 1-phenyl-3-(pyridin-4-yl)thiourea derivative (1)

Appropriate amounts of pyridine-2-ylamine (1 eq) and phenyl isothiocyanate (1 eq) were dissolved in ethanol and refluxed for 3-4 hours. The end of the reaction was checked by thin layer chromatography. After cooling, the solid was filtered and recrystallised with ethanol.

Synthesis of final compound 4-(4-chorophenyl)-N-phenyl-3-(pyridin-4-yl)thiazol-2(3H)-imine (2)

1-Phenyl-3-(pyridin-4-yl)thiourea derivative (1 eq) and 2-bromo-4'-chloroacetophenone (1 eq) were dissolved in ethanol and refluxed for 4 to 5 hours. The end of the reaction was checked by thin layer chromatography. The solid was cooled, filtered and recrystallized in ethanol [29, 30].

4-(4-Chorophenyl)-N-phenyl-3-(pyridin-4-yl) thiazol-2(3H)-imine (2)

Yield: 60%. M.p. 285-288°C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 7.20-7.22 (3H, m, Ar-H), 7.35-

7.42 (5H, m, Ar-H), 7.51-7.53 (4H, m, Ar-H), 7.59-7.61 (2H, m, Ar-H). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 105.51, 121.85, 124.79, 128.22, 128.93, 129.28, 129.59, 130.32, 130.60, 130.66, 130.94, 131.57, 132.50, 134.60, 134.85, 140.59, 168.76.

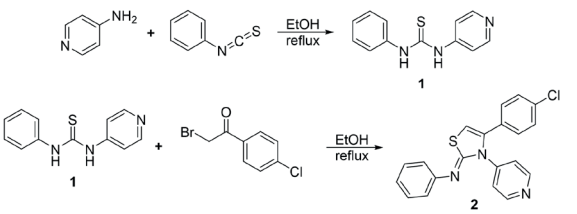
2.2. *In Silico* Studies

SwissADME and pkCSM-pharmacokinetics software programmes were used to study the physicochemical properties of the compound **2**. SwissADME software [31] and SwissTargetPrediction software [32, 33] were used to search the druglikeness and possible bioactivity profile of the compound **2**. Finally, the potential toxicity of our molecule was determined using the pkCSM pharmacokinetics software [34].

3. RESULTS AND DISCUSSIONS

3.1. Chemistry

In this study, we have synthesised (Scheme 1) novel 4-(4-chlorophenyl)-*N*-phenyl-3-(pyridin-4-yl)thiazol-2(*3H*)-imine. 1-Phenyl-3-(pyridin-4-yl) thiourea derivative synthesized from pyridin-2-amine and thiourea. 1-Phenyl-3-(pyridin-4-yl) thiourea and 2-bromo-4'-chloroacetophenone were



Scheme 1. General procedure for the synthesis of the final product 4-(4-chlorophenyl)-*N*-phenyl-3-(pyridin-4-yl) thiazol-2(*3H*)-imine (**2**)

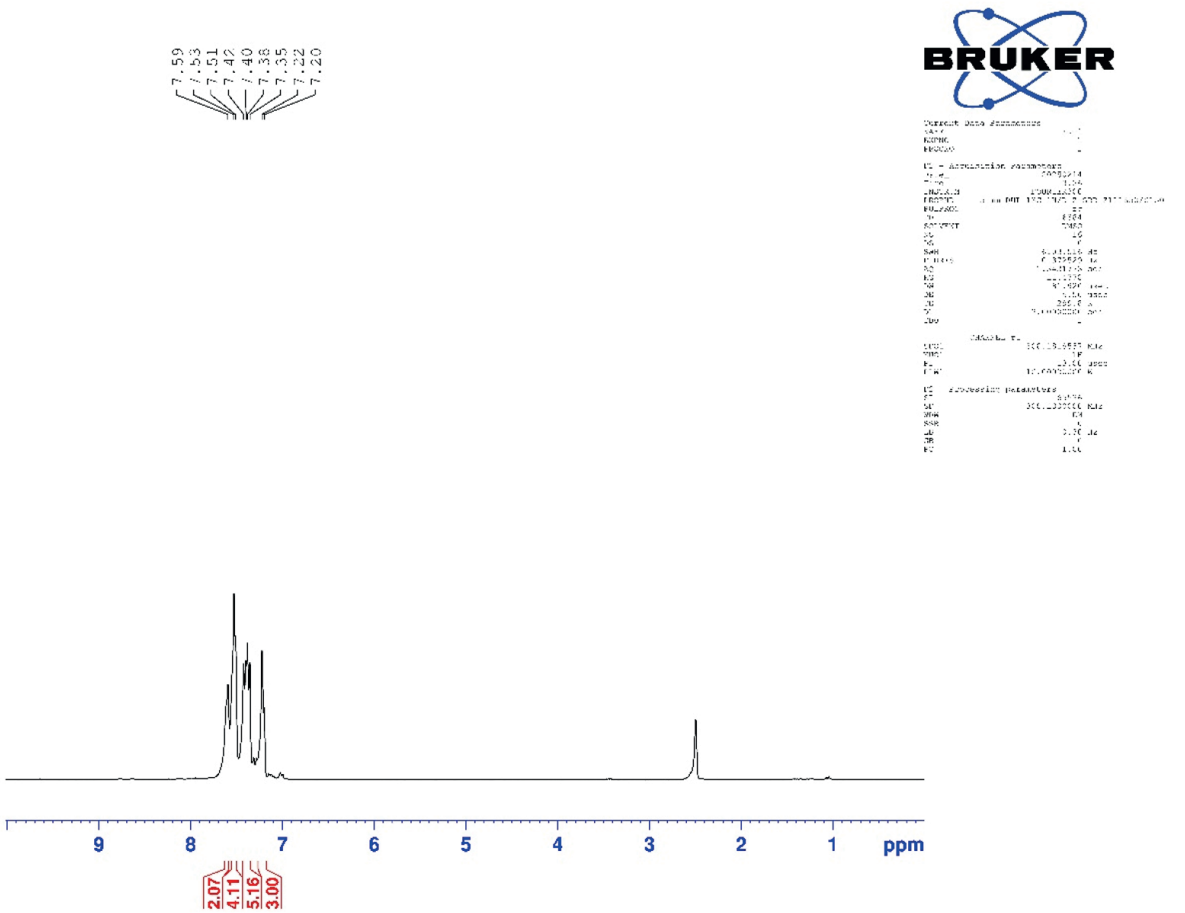


Figure 2. ^{1}H-NMR spectrum of compound **2**

reacted as described in the literature [29] to give the final product (**2**). To confirm the structure of compound **2**, various spectral analysis methods were used. ^{1}H-NMR, ^{13}C-NMR and 2D-NMR spectra were acquired and evaluated.

Compound **2** contains benzene, pyridine and thiazole rings in its structure. Aromatic -C-H proton peaks at 7.20-7.59 ppm were observed in the ^{1}H NMR spectra as shown in Figure 2. There are no aliphatic -C-H protons in the structure, therefore, there is no peak in the spectrum between 2.00-5.00 ppm. In the ^{13}C-NMR spectrum, carbon atoms were seen in the expected region, around 105.51-140.59 ppm (Figure 3).

Compound **2** was subjected to advanced 2D NMR (HSQC, NOESY, HMBC) studies. Firstly, the data obtained from the ^{1}H-NMR and ^{13}C-NMR spectra were analysed. Then, in order to match the carbons containing hydrogen(s), the HSQC spectrum was merged with the ^{1}H and ^{13}C NMR spectra. The HMBC data gives the spectrum of the carbons on the F1 axis, and the protons on the F2 axis, as in Figure 4. Then, using the data given, all the carbons are matched with the protons. According to HBMC spectrum at 168 ppm, there should be a single carbon interaction; this peak, compared with the HSQC spectrum and peaks are matched with one another. At 7.19 ppm on the F2 axis and at 105 ppm on the F1 axis, there were two peaks belonging to the thiazole ring in accordance with HSQC spectrum (Figure 5). These findings are proof that our ring has closed as designed.

3.2. *In Silico* Studies

The pharmacokinetic properties of the final compound were investigated using SwissADME and pkCSM pharmacokinetics software. Physicochemical properties, water solubility, pharmacokinetic properties (absorption, distribution, metabolism and excretion) and also druglikeness were calculated

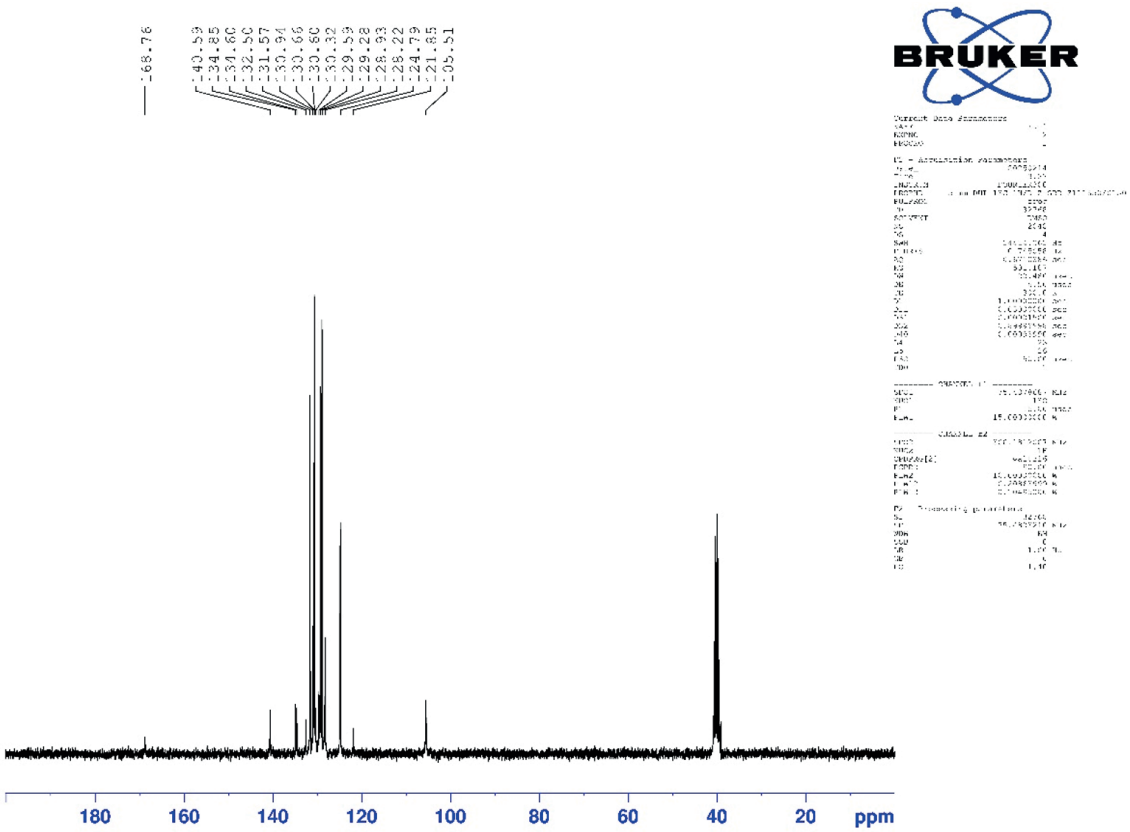


Figure 3. ^{13}C-NMR spectrum of compound **2**

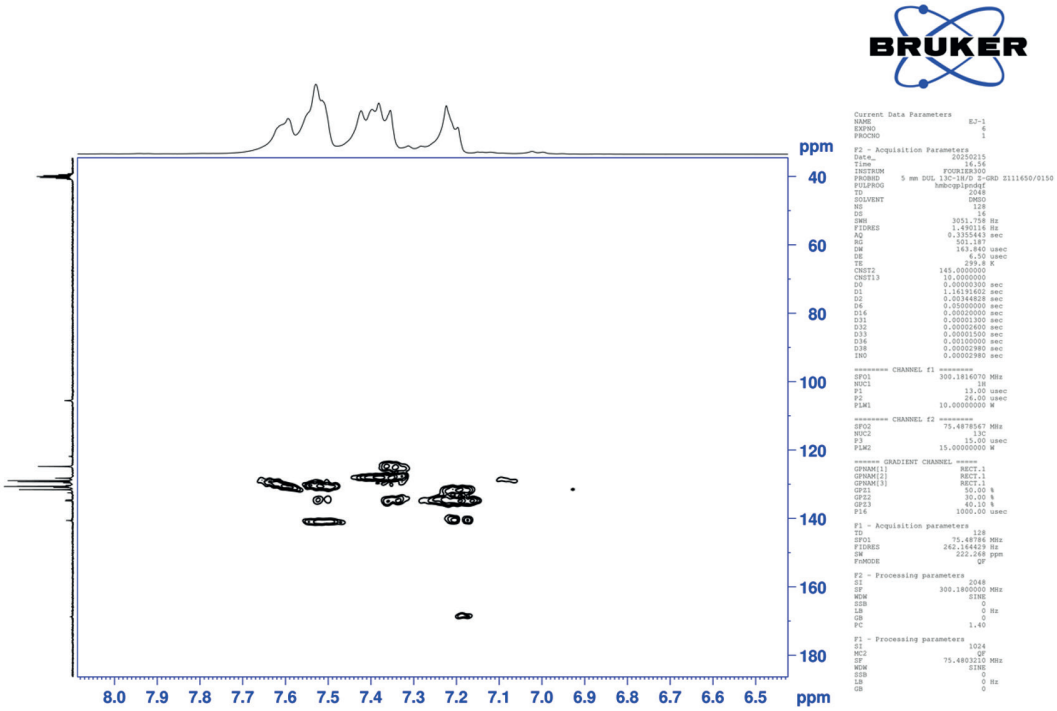


Figure 4. HBMC spectrum of compound **2**

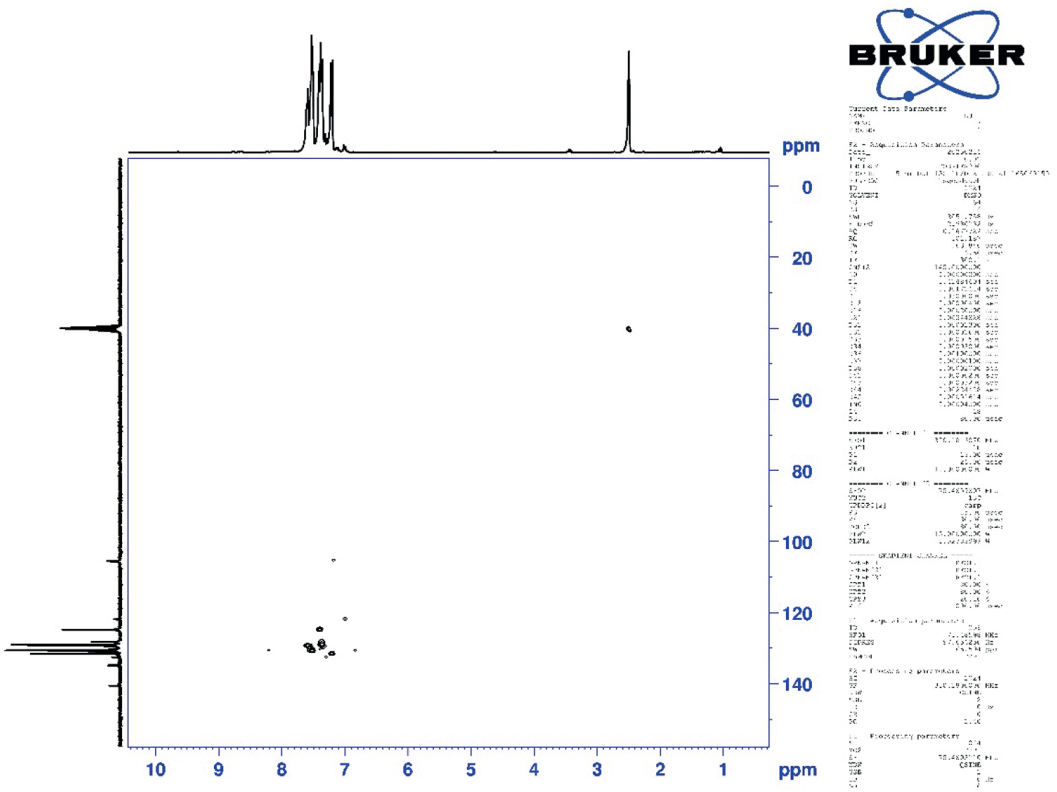


Figure 5. HSQC spectrum of compound **2**

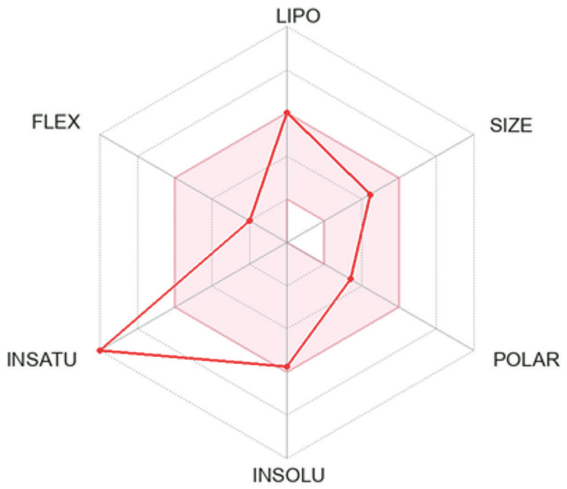


Figure 6. Physico-chemical field demonstration of the structure of compound **2** for the oral bioavailability

theoretically. Compound **2** has two hydrogen bond acceptor centres but no hydrogen bond donor centre. The topological polar surface area (TPSA) was 58.42 Å and the lipophilicity (cLogP) averaged over all five predictions was 4.75, close to the best range for these parameters. The solubility class of compound **2** in water was found to be moderately soluble according to logS value of 5.75 . Compound **2** can inhibit CYP1A2, CYP2C19, CYP2C9 and CYP3A4 enzymes. These features may lead to drug-drug interactions with our compound. Based on Lipinski's Rule of Five, compound **2** has a probability of being a orally bioavailable drug (Figure 6). In addition to Lipinski, there is also no violation according to the rules of Ghose, Veber and Egan.

To predict the gastrointestinal absorption and brain penetration of compound **2**, the boiled-egg plot between water partition coefficient (WLogP) and TPSA was also studied in SwissADME. As shown in Figure 7, Compound **2** was predicted to permeate the BBB and also to enter the gastrointestinal tract. Furthermore, red dots indicate molecules predicted not to be cleared by P-glycoprotein.

SwissTarget prediction software was used to identify the target prediction of the final compounds. The best 15 targets were predicted and the commonly predicted target regions are shown in Figure 8. Compound **2** was found to target phosphodiesterase, electrochemical transporter, lyase and enzyme with a percentage of 33.3%, 20.0%, 13.3% and 13.3%, respectively. Also with the same percentage of 6.7% compound **2** target on membrane receptor, writer and family A G protein-coupled receptor. With this, our compound may have a high level of attraction to the predicted targets.

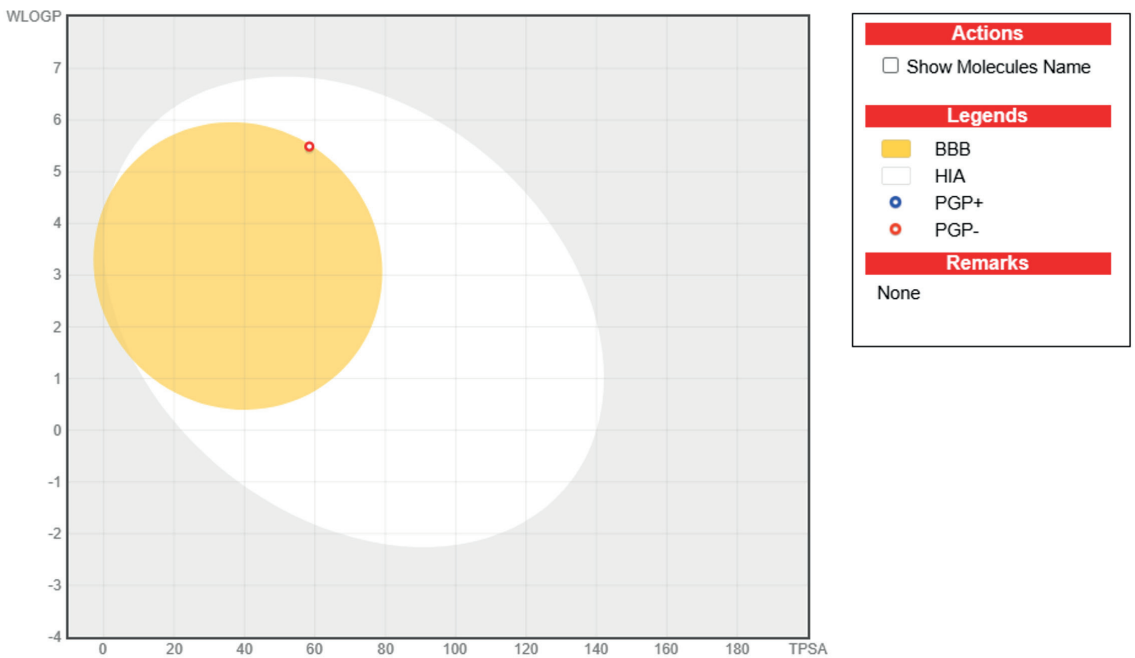


Figure 7. Boiled-egg screening of compound **2**

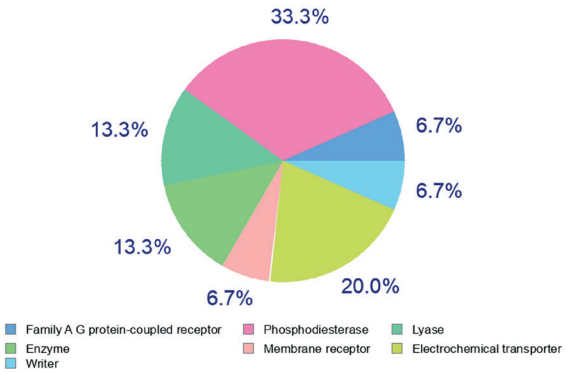


Figure 8. Pie chart showing the SwissTarget prediction of compound **2**

Table 1. Predicted toxic profiles using pkCSM software for compound **2**

	Compound 2
AMES toxicity (Yes/No)	Yes
Max. tolerated dose (human) (log mg/kg/day)	0.224
hERG I inhibitor (Yes/No)	No
hERG II inhibitor (Yes/No)	No
Oral Rat Acute Toxicity (LD_{50}) (mol/kg)	1.853
Oral Rat Chronic Toxicity (log mg/kg_bw/day)	0.359
Hepatotoxicity (Yes/No)	No
Skin Sensitisation (Yes/No)	No
T.Pyriformis toxicity (log ug/L)	0.304
Minnow toxicity (log mM)	0.355

The pkCSM software was also used to predict the toxicity profiles of the compounds and the results are shown in Table 1. The website can provide details of the toxicological effects in the following areas: AMES toxicity, human maximum tolerated dose, hERG-I inhibitor, hERG-II inhibitor, LD_{50} (lethal dose), chronic oral toxicity in rats, hepatotoxicity, skin toxicity, T. pyriformis toxicity and minnow toxicity. The results showed that our compound does not have any hepatotoxicity and is not skin sensitising either. However, it does have AMES toxicity, which means that it has a mutagenic effect. They don't inhibit hERG-I and hERG-II, which is a favorable finding which gives a non-cardiotoxic profile to the compounds. The acute oral toxicity (LD_{50}) of the compounds to rats was found to be 1.853 mol/kg, while the chronic oral toxicity (LOAEL) to rats was found to be 0.359 log mg/kg_bw/day. Toxicity to *T. pyriformis* has been reported in the range of 0.304 log µg/L.

4. CONCLUSION

A new 4-(4-chlorophenyl)-*N*-phenyl-3-(pyridin-4-yl)thiazol-2(*3H*)-imine derivative (**2**) was prepared following the literature knowledge of thiazole ring system synthesis methodology. Spectra of 2D NMR proved that our final product synthesized according to the synthesis scheme. The thiazole ring was closed from the thiourea structure as expected (Scheme 1). The final compound was evaluated for its physicochemical/pharmacokinetic properties as well as druglikeness properties. Lipinski's rule of five and other rules such as Ghose, Veber or Egan were not violated by compound **2**. Compound **2**'s lipophilicity is in the desired range of five different parameters. It was also predicted that compound **2** would not be hepatotoxic and would not be irritating to the skin. This synthesized compound needs to be investigated as a potential antifungal drug candidate because of the structure of the compund similar to the drugs against fungal infections. Further studies will include synthesizing new derivatives, *in vitro* and *in silico* studies.

Ethical approval

Ethics committee approval is not required as there are no in vivo or clinical studies.

Author contribution

Concept, L.Y.; Supervision, L.Y.; Methods, A.Z.K., and L.Y.; Data acquisition and/or processing, A.Z.K., L.Y.; Analysis and/or interpretation, A.Z.K.; Investigation, L.Y.; Writing - original draft preparation, A.Z.K., L.Y.; Critical review, L.Y. The

published version of the manuscript was read and approved by all authors.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Research Article

Antioxidant, anti-acetylcholinesterase potentials, ADME estimations and molecular docking studies of green algae extracts

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ABSTRACT

Algae have become the center of attention due to their strong antioxidants and enzyme-inhibitory activities. In this study, green algae (*Enteromorpha linza*) extracts obtained using acetone, hexane and methanol were investigated. In the study, antioxidant properties, anti-acetylcholinesterase (AChE) potential, ADME estimations and molecular docking analyses of green algae extracts were investigated. The best binding position was obtained by docking sirsimaritin, daidzein, kaempferol, morin and myricetin to the active site of acetylcholinesterase receptor. Docking score values were calculated as -10.0, -10.3, -9.9, -9.8 and -9.8 kcal/mol, respectively. Experimental analysis revealed that the extracts showed inhibitory activity against acetylcholinesterase enzyme. Acetone and hexane extract showed good inhibition performance with IC_{50} values of 0.0379 mg/ml and 0.0414 mg/ml, respectively. The IC_{50} value for methanol extract was determined as 0.997 mg/ml. When the antioxidant activity results of the extracts were evaluated in terms of both DPPH and ABTS radical scavenging capacities, it was revealed that the acetone-based extract had a higher radical scavenging capacity (DPPH: 17.48%, ABTS: 83.58%) compared to the extracts obtained with other solvents. In general, the obtained results revealed that the green algae examined can be used as a source of natural agents beneficial for human health.

Keywords: AChE, Algae, Antioxidant, Inhibition, Molecular docking

1. INTRODUCTION

In recent years, interest in bioactive compounds from marine resources has increased significantly due to their positive effects on health. Macroalgae (seaweeds) in particular stand out as a rich source of various bioactive substances. The cell walls of these algae contain different types of sulfated polysaccharides, and these compounds show a wide range of biological activities such as anti-coagulant, antiviral, antioxidant, anticancer and immunomodulatory effects. Therefore, they offer significant potential for the nutraceutical, pharmaceutical and cosmeceutical industries [1].

Algae are diverse photosynthetic organisms that live in water and encompass thousands of species. In general, seaweeds are divided into two main classes: microalgae and macroalgae (often called seaweeds). Macroalgae are further divided into three subgroups: green algae (Chlorophyta), brown algae (Phaeophyta), and red algae (Rhodophyta). These

groups differ in species diversity and chemical composition [2]. Green algae, one of the three main groups of macroalgae, are widespread in the marine environment and are distributed worldwide [3]. The genus *Enteromorpha* (also known as *Ulva*), a group of green algae, is an important macroalgae causing green tides. *Enteromorpha* species are notable for their high tolerance to environmental stresses and rapid growth rates [4,5]. *Enteromorpha* species are therefore seen as a promising source of biomass for various industrial applications [6,7]. *Enteromorpha* species contain a variety of nutrients such as carbohydrates, proteins, fats, minerals and vitamins. Studies on *Enteromorpha* extracts and isolates have shown that these algae have antioxidant, antimicrobial, anti-ageing, anti-radiation and moisturizing properties [5,8].

Alzheimer's disease (AD) is a neurodegenerative disease characterized by atypical behavioral symptoms, memory impairment, cognitive decline, and intellectual disability, frequently seen in the ageing population [9,10]. Alzheimer's disease (AD) is pathologically defined by neuronal degeneration, the presence of senile plaques located interstitially between neurons, and changes in synaptic architecture [11]. The aetiology of AD highlights the critical function of acetylcholinesterase (AChE) within the central nervous system. The primary role of AChE involves the enzymatic hydrolysis of acetylcholine (ACh) into acetate ($CH3COO^-$) and choline (Ch) [12,13]. When AChE content is high and its activity is strong, ACh content decreases rapidly. In this case, neuronal damage occurs, and AD occurs. Therefore, oral drugs that can inhibit the existing AChE content in the brain can easily alleviate the symptoms of AD patients [14]. Therefore, the use of AChE inhibitors (AChEIs) for cholinergic degradation of ACh offers a promising and effective approach in the treatment of Alzheimer's disease (AD) [15].

Molecular Docking is a computational method widely used in drug design. This technique helps to introduce new molecules into the field by assessing the strength of interactions between the molecules under study and biological systems such as enzymes, while providing mechanistic insights into chemical interactions [16,17].

During normal cellular metabolism in living organisms, reactive oxygen species (ROS) are produced, which can be detrimental to essential biomolecules such as lipids, carbohydrates, nucleic acids, and proteins [18–20]. Additionally, ROS serve as a primary immune defence mechanism in all living beings and are implicated in numerous diseases [21,22]. Studies have revealed that oxidative stress and reactive oxygen species (ROS) are key determinants contributing to numerous chronic conditions, including cancer, immunodeficiency syndrome, age-related pathologies, cardiovascular diseases, arteriosclerosis, diabetes, and obesity [23,24]. Consequently, inhibiting the formation of ROS may be crucial in reducing the incidence of chronic diseases [25].

Nowadays, the development of natural and reliable treatment strategies for neurodegenerative diseases and oxidative stress-related pathologies has been receiving increasing attention in the literature. In this context, the rich bioactive compound portfolio of marine-derived natural products draws attention with their potential that has not yet been fully explored. In light of current studies, it is thought that a comprehensive evaluation of molecular interactions and pharmacokinetic properties of green algae extracts will better determine the place of natural agents in therapeutic applications. This study aims to provide new perspectives on the biological activities of this natural resource by examining the antioxidant and acetylcholinesterase inhibition potentials of green algae extracts through the integration of experimental analyses and computational methods.

2. MATERIALS AND METHODS

2.1. Preparation of Extracts

The green algae (*Enteromorpha linza)* were collected from Izmir, Türkiye. The collected sample was brought to the laboratory in plastic bags containing seawater to prevent evaporation. The algae were washed thoroughly with tap water and distilled water to remove surface particles and epiphytes and dried in the shade for 5 days. They were dried in an oven at 50 °C until constant weight was achieved. The dried algae were ground into fine powder using an electric mixer. They were mixed with acetone (E-

A), hexane (E-H) and methanol (E-M) (solid/liquid ratio 1:10 w/v) as solvents. The mixture was placed on a magnetic stirrer and stirred for 2 h. Then, it was filtered using a Whatman No. 1 filter. The filtrate obtained was dried and stored at 4 °C until needed [26,27].

2.2. Molecular Docking Studies

Molecular docking studies were carried out using the AutoDock Vina tool and UCSF Chimera software [28–30]. Cholinesterase and bioactive phytochemicals (cirsimaritin, daidzein, kaempferol, morin and myricetin) were evaluated for molecular docking studies. Acetylcholinesterase (PDB:4M0E) Structure of human acetylcholinesterase in complex with dihydrotanshinone I Method: X-RAY diffraction resolution: 2.00 Å) receptor was retrieved from the RCSB (https://www.rcsb.org/) protein data bank. After minimization, the grid box resolution for docking along the x, y and z axes was set to the binding region. Results were recorded after docking analysis [31,32]. Protein-ligand interactions were evaluated in the https://proteins.plus/ and amino acids were labelled. Finally, both 2D and 3D structures of the protein-ligand interface were shown. The PoseEdit system employs the PoseView algorithm and the InteractionDrawer JavaScript library (https://github.com/rareylab/InteractionDrawer) to automatically generate highly detailed 2D and 3D diagrams illustrating ligand interactions. The structural representations adhere to IUPAC guidelines. The system visualizes various computed interactions between the ligand and nucleic acids, amino acids, and metals, including hydrogen bonds, cation-pi interactions, pi-stackings, ionic interactions, and metal interactions, using colored dashed lines. Additionally, hydrophobic contacts are represented by green splines with labels [33].

2.3. ADME Analysis

Swiss ADME online web tool (http://www.swissadme.ch/) was used to perform ADME analysis of cirsimaritin, daidzein, kaempferol, morin and myricetin compounds [34]. ChemDraw was utilized to generate SMILES data for these compounds. Subsequently, various physicochemical properties were evaluated, including lipophilicity, drug-likeness, pharmacokinetics, topological polar surface area (TPSA), the number of rotatable bonds, and any violations of Lipinski's rule of five.

2.4. Acetylcholinesterase (AChE) Inhibition Activity

The inhibitory effect of green algae on acetylcholinesterase (AChE) was evaluated [35]. Briefly, 100 µL of 1 M Tris-HCl buffer (pH 8.0) was combined with 10 µL AChE solution and 10 µL extract and the total volume was brought to 900 mL with distilled water. It was then incubated at 25 °C for 5 min. Then, 50 µL of acetylthiocholine iodide (AChI) and 50 µL of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) were added to the reaction medium. The resulting mixture was incubated for another 5 min at 25 °C. A control reaction was performed using an equivalent volume of dimethyl sulfoxide (DMSO) instead of the sample. Absorbance measurement was carried out at 405 nm in a spectrophotometer. The percentage of inhibition was determined according to the following formula (1):

$$Inhibiton\ (\%) = \frac{(A_c - A_s)}{A_c}\ X\ 100 \qquad (1)$$

Where A_c is the absorbance value of the control and A_s is the absorbance value of the sample.

2.5. DPPH Radical Scavenging Activity

To study the antioxidant potential of the crude extract of green algae, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (RSA) was performed with slight modifications to the previously reported method [36]. The experiment was carried out by adding 100 µL (1 mg/mL) of algae extract to 900 µL of DPPH (0.1 mM) mixture. The reaction mixture was then incubated at room temperature for 30 min in the dark to record absorbance (517 nm) readings on a spectrophotometer. 1 mL of DPPH was used as control. DPPH radical scavenging activity was calculated according to the following formula (2):

$$Radical\ scavenging\ activity(\%) = \frac{(A_c - A_s)}{A_c}\ X\ 100 \qquad (2)$$

Where A_c is the absorbance value of the control and A_s is the absorbance value of the extracts.

2.6. ABTS Radical Scavenging Activity

2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of green algae was determined according to the previously described method with minor revisions [5]. Briefly, ABTS (7 mM) was mixed with potassium persulfate (2.45 mM) in the dark at room temperature for 16 h to form ABTS radicals. The solution was then diluted with phosphate buffer (pH = 7.4) until the absorbance at 734 nm was 0.70 ± 0.02. 5 µL of sample solution prepared at a concentration of 1 mg/ml was reacted with 995 µL of ABTS solution and the absorbance of the mixture was measured at 734 nm.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield (Ey)

To determine the extraction yield (Ey), the percentage of the ratio between the extracted mass and the dry sample mass was calculated. Acetone-extract (E-A), hexane-extract (E-H) and methanol-extract (E-M) had values of 13.5, 11.4, 14.7%, respectively. These values revealed that the methanol extract had a higher yield.

3.2. Molecular Docking

A crucial computational technique for examining the structural and dynamic characteristics of biomolecular systems is molecular docking. This method, extensively utilized in drug discovery and biotechnology research, enables the investigation of how small molecules, known as ligands, interact with the active sites of target proteins and the potential impacts of these interactions on biological systems [37]. Molecular docking analyses evaluate ligand-protein interactions in terms of binding energies, with the lowest docking score indicating the highest binding affinity [38]. The composition of the extract was determined based on previously published data [39–41]. The best binding pose was obtained by docking cirsimaritin, daidzein, kaempferol, morin and myricetin into the active site of acetylcholinesterase (PDB: 4M0E) receptor. Docking score values were calculated as -10.0, -10.3, -9.9, -9.8 and -9.8 kcal/mol, respectively.

Chemical interactions between molecules and proteins typically involve conventional hydrogen bonds, pi-pi-shaped bonds, pi-alkyl bonds, pi-anion bonds, carbon-hydrogen bonds, pi-pi stacked bonds, and pi-sulfur bonds. The 2D-3D structures of hydrogen bonds, Pi-pi interaction, ionic interaction, cation-pi interaction and hydrophobic interactions are shown in Figure 1. 4M0E protein formed 1 hydrogen bond with Cirsimaritin, 3 with Daidzein, 2 with Kaempferol, 2 with Morin and 3 with Myricetin. These bonds formed are Phe287, Ser822, Tyr866, Tyr870, Phe287, Tyr329, Phe824, Arg825, Tyr1030, Arg 1061 and Ala 1053, respectively. All other interactions are given in Figure 1. Hydrogen bonds provide specific and directional interactions between the ligand and the protein. This facilitates the localization of the ligand to the correct binding site (active site, binding pocket, etc.) on the protein. The aromatic rings of the ligand can contribute to binding stability by forming π-π interactions with aromatic amino acids in the protein. Specific binding is critical for accuracy in biological processes. The colored spirals in the protein represent α-helix regions. These regions often provide structural stability and can be important in ligand binding [42].

Several compounds, including cirsimaritin, daidzein, kaempferol, morin, and myricetin, demonstrated inhibitory effects on various enzymes such as CYP1A2, CYP2C9, CYP2D6, CYP2C19, and CYP3A4. The BOILED-Egg graph, a predictive model based on molecular lipophilicity and polarity, was utilized to assess the likelihood of gastrointestinal absorption and blood-brain barrier (BBB) penetration. This graph is divided into three distinct areas. As illustrated in Figure 2, the white section of the BOILED-Egg graph (albumin) signifies molecules with high potential for gastrointestinal (GI) absorption, while the yellow area represents possible BBB permeability. Molecules with low GI absorption and minimal brain penetration are depicted in the gray region. The presence of cirsimaritin, kaempferol, and morin in the white area suggests a high likelihood of GI absorption, indicating that these compounds may have increased bioavailability when taken orally.

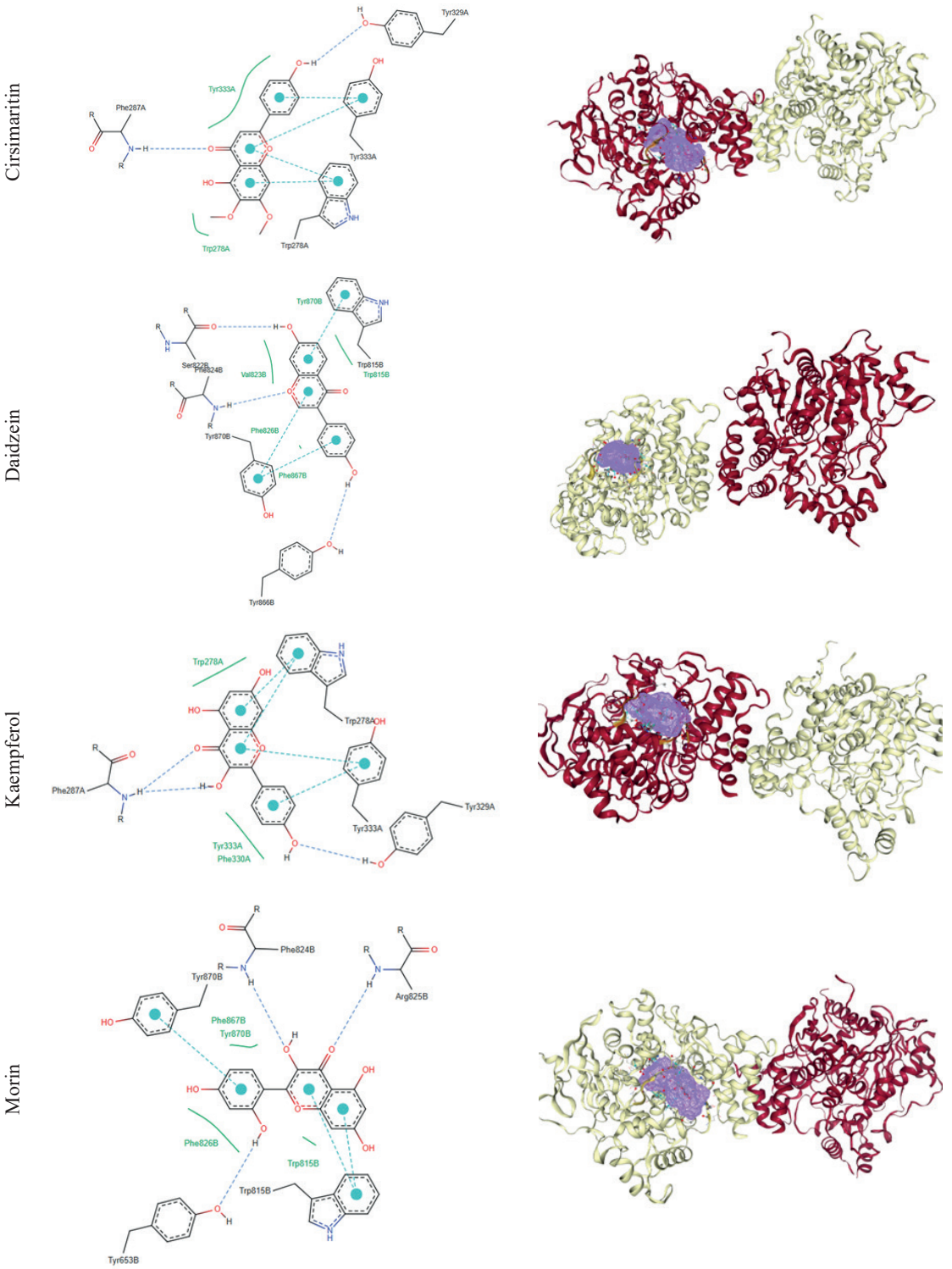

Figure 1. The 2D and 3D binding mode of Cirsimaritin, Daidzein, Kaempferol, Morin and Myricetin ligands with AChE (4M0E)

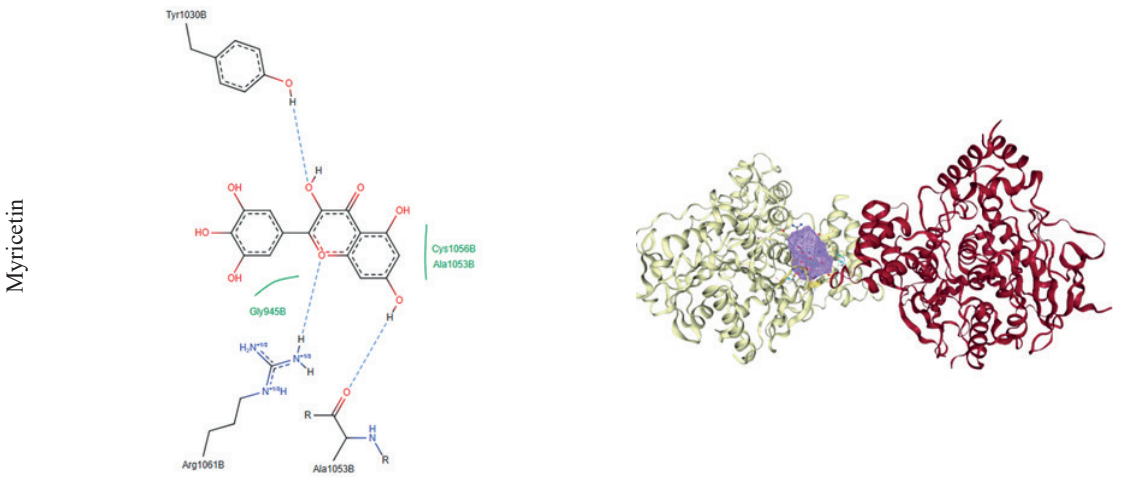

Figure 1. The 2D and 3D binding mode of Cirsimaritin, Daidzein, Kaempferol, Morin and Myricetin ligands with AChE (4M0E) (Continued)

ADME calculations are performed for the use of molecules as drugs in human metabolism. According to chemical parameters such as molar masses of molecules, dipole moment of molecules, hydrogen bonds given and taken by molecules and biological parameters such as absorption of molecules through intestinal and blood barriers, absorption through the skin or numerical values of oral absorption, it is seen that there is no harm in using them as drugs. The presence of different groups in cirsimaritin, daidzein, kaempferol, morin and myricetin suggests that their activity can be enhanced by changing their physicochemical properties and pharmacokinetic parameters to increase their bioavailability and metabolic stability as well as their binding affinity to receptors.

The ADME properties of selected flavonoids provide insights into their pharmacokinetic potential (Table 1). Gastrointestinal (GI) absorption is high for all compounds except for myricetin, which exhibits lower absorption likely due to its high total polar surface area (TPSA = 151.59 Å). In terms of blood-brain barrier (BBB) permeability, only daidzein shows the ability to penetrate the central nervous system, which can be attributed to its lower TPSA (70.67 Å) and moderate lipophilicity (iLOGP = 1.77, XLOGP3 = 2.47).

Regarding metabolism, all flavonoids inhibit CYP1A2 and CYP3A4, suggesting potential drug-drug interactions. However, only cirsimaritin inhibits CYP2C9, which is involved in the metabolism of several anti-inflammatory drugs. Furthermore, all flavonoids except myricetin inhibit CYP2D6, which is responsible for metabolizing numerous psychoactive drugs.

Lipophilicity indices (iLOGP, XLOGP3, and WLOGP) suggest that cirsimaritin is the most lipophilic compound, while myricetin is the least. This is consistent with their respective skin permeation coefficients (Log Kp), where cirsimaritin exhibits the highest permeability (-5.86 cm/s) and myricetin the lowest (-7.40 cm/s). These results indicate that cirsimaritin might be better suited for transdermal delivery systems.

All compounds comply with Lipinski's rule of five, indicating good oral bioavailability. However, myricetin does not satisfy Veber, Egan, or Muegge rules, which may imply limitations in its bioavailability and drug-likeness.

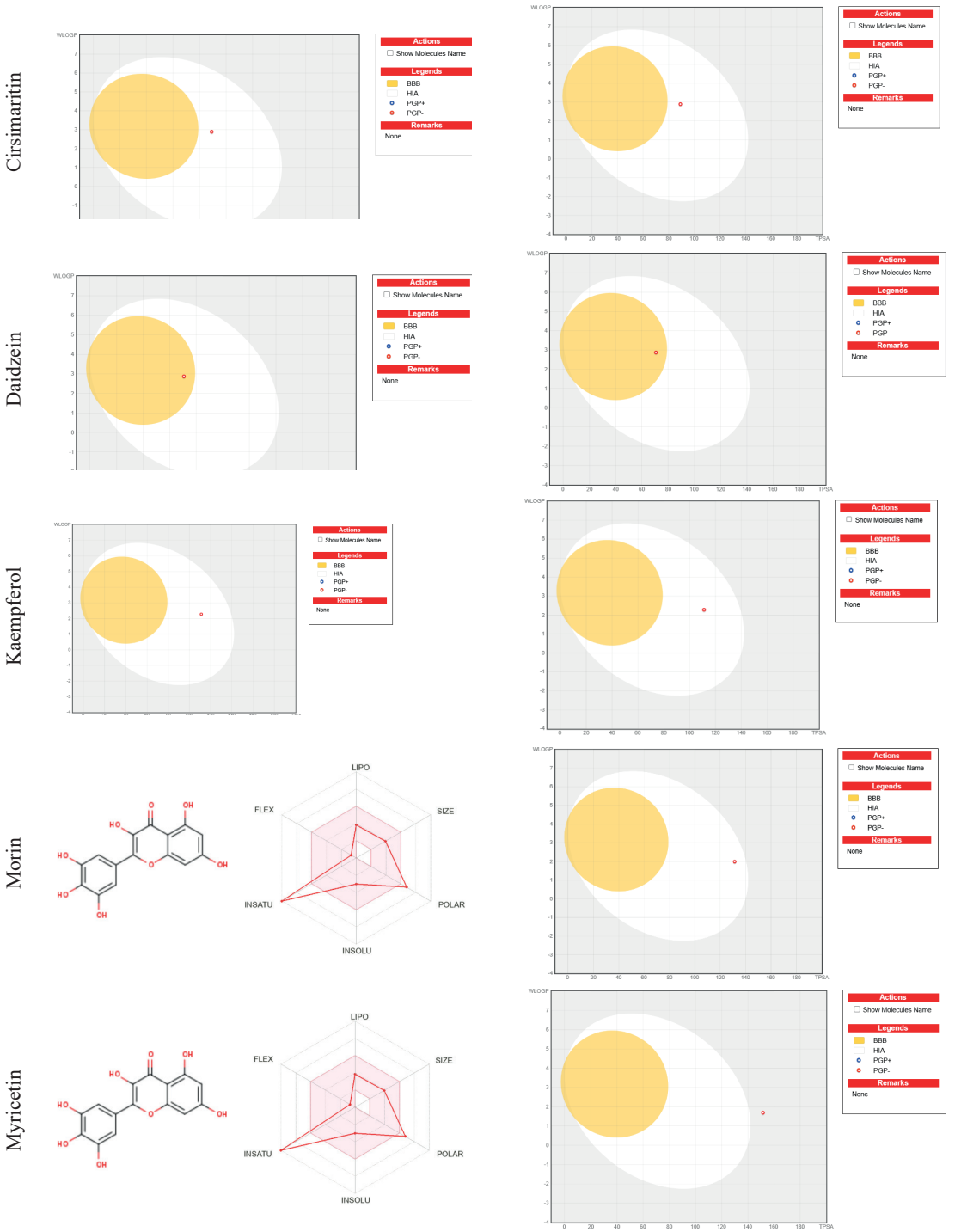

Figure 2. BOILED-Egg chart

Table 1. Physicochemical and ADME properties of cirsimaritin, daidzein, kaempferol, morin, and myricetin

	Cirsimaritin	Daidzein	Kaempferol	Morin	Myricetin
Molecular weight	314.29 g/mol	254.24 g/mol	286.24 g/mol	302.24 g/mol	318.24 g/mol
Num. H-bond acceptors	6	4	6	7	8
TPSA	89.13 Å	70.67 Å	111.13 Å	131.36 Å	151.59 Å
iLOGP	2.56	1.77	1.70	1.47	1.08
(XLOGP3)	3.32	2.47	1.90	1.54	1.18
WLOGP	2.89	2.87	2.28	1.99	1.69
MLOGP	0.47	1.08	-0.03	-0.56	-1.08
SILICOS-IT	3.07	3.02	2.03	1.54	1.06
ESOL	-4.20	-3.53	-3.31	-3.16	-3.01
GI absorption	High	High	High	High	Low
BBB permeant	No	Yes	No	No	No
CYP1A2 inhibitor	Yes	Yes	Yes	Yes	Yes
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	Yes	No	No	No	No
CYP2D6 inhibitor	Yes	Yes	Yes	Yes	No
CYP3A4 inhibitor	Yes	Yes	Yes	Yes	Yes
Log K_p (skin permeation)	-5.86 cm/s	-6.10 cm/s	-6.70 cm/s	-7.05 cm/s	-7.40 cm/s
Lipinski	Yes	Yes	Yes	Yes	Yes
Ghose	Yes	Yes	Yes	Yes	Yes
Veber	Yes	Yes	Yes	Yes	No
Egan	Yes	Yes	Yes	Yes	No
Muegge	Yes	Yes	Yes	Yes	No
Bioavailability Score	0.55	0.55	0.55	0.55	0.55

3.3. Acetylcholinesterase (AChE) Inhibition Activity

Acetylcholinesterase is an enzyme found in nerve synapses. Its primary function is to break down the neurotransmitter acetylcholine [43]. In the present study, the acetone-based green algae *(Enteromorpha linza*) extract obtained showed the highest AChE inhibition with an IC_{50} value equal to 0.0379 mg/mL (Figure 3). The hexane extract showed inhibition close to the acetone extract and had an IC_{50} value of 0.0414 mg/mL (Figure 4). Methanol-based extract had the lowest inhibition value and IC_{50} value was calculated as 0.997 mg/mL (Figure 5).

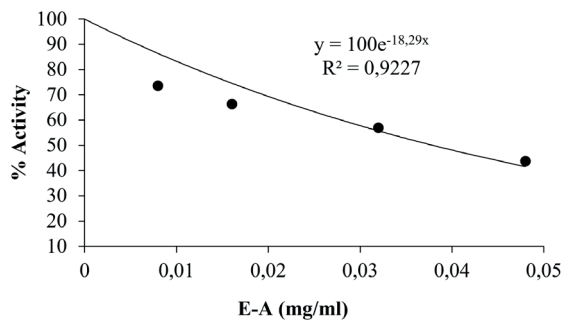

Figure 3. Effect of acetone-based extract (E-A) concentration on the activity of the AChE

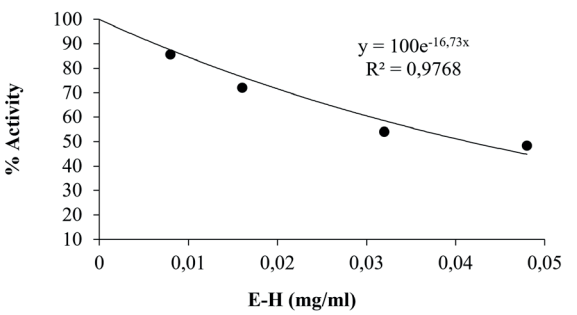

Figure 4. Effect of hexane-based extract (E-H) concentration on the activity of the AChE

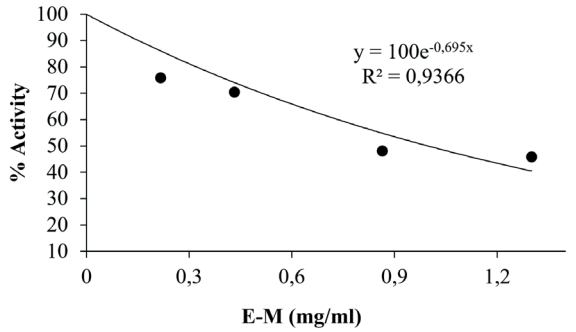

Figure 5. Effect of methanol-based extract (E-M) concentration on the activity of the AChE

3.4. Antioxidant Test

Studying the antioxidant properties of natural substances is an important first step in evaluating potential new therapeutic drugs and understanding their various functions in different diseases [44]. Table 2 summarizes the radical scavenging capacities of green algae extracts. It was found that the algae extracts showed variable radical scavenging abilities on DPPH and ABTS. The DPPH radical scavenging capacity of extracts with 100 µg/mL concentration showed the highest antioxidant property with 17.48 % for acetone extract. The hexane and methanol extracts showed similar radical scavenging capacity (11.89 and 11.48 %, respectively). When the extracts with 5 µg/mL concentration were evaluated in terms of ABTS radical scavenging capacity, acetone extract showed the highest antioxidant capacity with 83.58 % radical scavenging. Then methanol extract showed 78.38 % and hexane extract showed 25.78 % ABTS radical scavenging capacity.

In a previous study, it was reported that the extracts obtained using different solvents such as acetone, hexane and methanol showed different DPPH and ABTS radical scavenging activities, as in our study [45]. DPPH and ABTS radical scavenging capacities were evaluated by calculating IC_{50} (µg/mL) values. The highest radical scavenging for DPPH was achieved by methanol: 35.1 ± 0.27, acetone: 38.3 ± 0.58, hexane: 66.0 ± 3.81. The highest radical removal for ABTS was methanol: 43.2 ± 0.24, acetone: 43.4 ± 0.39, hexane: 91.4 ± 0.39, respectively. Unlike our study, the highest radical scavenging capacity was found in methanol extract. The differences between the antioxidant activities of the extracts may be due to differences in the composition or amounts of antioxidant components in the extracts.

Table 2. DPPH and ABTS free radical-scavenging activity (inhibition %) of extracts

Extracts	DPPH	ABTS
Aceton Extract (E-A)	17.48	83.58
Hexane Extract (H-A)	11.89	25.78
Methanol Extract (M-A)	11.48	78.38

4. CONCLUSION

The present study determined that green algae extract exhibited significant biological activities such as anti-acetylcholinesterase and antioxidant properties. In particular, acetone (IC_{50}: 0.0379 mg/mL) and hexane (IC_{50}: 0.0414 mg/mL) based extracts were determined to have inhibitory potential on acetylcholinesterase enzyme. Molecular docking analyses supported these findings by showing interactions between bioactive compounds and AChE. Furthermore, ADME evaluations revealed that the identified bioactive compounds exhibited promising pharmacokinetic properties, enhancing their potential for drug development. Among the selected flavonoids, daidzein appears to have the best central nervous system accessibility, while cirsimaritin demonstrates the highest permeability and lipophilicity. The strong CYP enzyme inhibition across all compounds suggests that these flavonoids could interact with other drugs metabolized by these pathways. Further experimental studies are required to validate these predictions and explore their pharmacological potential. As a result of DPPH and ABTS radical scavenging analyses, acetone extract was determined to have higher antioxidant capacity (DPPH: 17.48%, ABTS: 83.58%) than hexane and methanol extracts. Overall, the obtained results indicate that green algae extract can be used for human benefit due to its promising biological activities.

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Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization, A.N., M.I. and Ş.B.; Methodology, A.N., M.I. and Ş.B.; Software, A.N.; Validation, A.N. and M.I.; Formal analysis, O.Ç., A.N. and M.I.; Investigation, O.Ç., A.N. and M.I.; Data curation, O.Ç., A.N. and M.I.; Writing—original draft preparation, O.Ç., A.N. and M.I.; Writing—review and editing, O.Ç., A.N. and M.I.; Visualization, O.Ç., A.N. and M.I.; Supervision, Ş.B. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Research Article

Evaluation of pharmacy technician training programs in different countries

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ABSTRACT

In recent years, there have been significant changes in pharmacy practices. There have been significant changes in pharmacy practice in recent years. These changes have been driven by the increase in chronic diseases and lifestyle-related illnesses. The changes in pharmacy practice have resulted in pharmacists being involved in a greater role in patient-centered health care and therefore requiring more time. In order to reduce this workload and provide pharmacists with the time they need; it is possible for pharmacists to work in coordination with pharmacy technicians. At this point, the competency of pharmacy technicians in their professions and the high quality of the education they receive play a key role in pharmacy management and pharmacy practices. In this study, the education of pharmacy technicians in other countries was evaluated. The similarity of pharmacy technician education is thought to make a significant contribution to the standardization of education. As a result of these studies, it is expected that the standardization of pharmacy technician education worldwide will contribute to the improvement of pharmacy practice and the quality of pharmacists and pharmacy health services.

Keywords: Pharmacy technician, Pharmacy technician education, Pharmacy management

1. INTRODUCTION

In the summary of the 2017 Pharmacy Technician Stakeholder Consensus, the agreed general definition of pharmacy support staff is as follows: Pharmacy support staff is a role that encompasses a wide range of roles, from staff with only various administrative tasks to staff who assist pharmacists in the performance of their professional tasks under the supervision of pharmacists (pharmacy assistants) [1]. In Türkiye, the definition of pharmacy technician was made in 2011; pharmacy technicians graduated from the pharmacy services program of vocational schools prepare prescribed medicines under the supervision of pharmacists and assist in pharmacy activities [2].

In recent years, there have been significant changes in pharmacy practice. The main reason for the changes is the increase in chronic diseases and lifestyle-related diseases in the society. Due to this increase, both community and hospital pharmacists assume more responsibility for patient-centered health care. The key role of pharmacists in providing both preventive and chronic health care has led to the transformation of pharmacy from dispensing medicines to providing health care. However, with the increasing demand for improved patient care, various problems arise for pharmacists, such as lack of time. In addressing these issues, the pharmacy technician's assistance to the pharmacist plays a key role [3]. In addition, the International Pharmaceutical Federation (FIP) argued in April 2024 advocated in April 2024 that investing in the pharmaceutical

workforce will benefit the development of health systems and contribute to achieving global health coverage by 2030, as previously stated in the United Nations Sustainable Development Goals [4].

Several studies have investigated the workforce support of pharmacy technicians to pharmacists. However, it is a fact that the scope of practice and work of pharmacy technicians is not clearly defined in many parts of the world as it is in our country. Furthermore, the practice scope of pharmacy technicians is reported to be a controversial issue. However, previous studies have shown that the support of pharmacy technicians to pharmacists will enable pharmacists to improve patient clinical care services. In addition, it has been reported that expanding their scope of practice with several tasks would be very beneficial for public health [5,6]. In addition, several studies have shown that pharmacists generally report that appropriately trained technicians add more value to the organization in which pharmacy technicians work. Furthermore, pharmacists have been reported to support expanding the pharmacy technician's role within certain limits [7,8]. Moreover, it has long been recognized that those who have been trained with a specific accreditation are more valued by their employers [9].

A survey of appropriately trained pharmacy technicians was conducted in the United States. In the surveys, 7 out of 17 reported an increase in their hourly wage after completing their certification program. Also, they reported feeling that their self-efficacy was higher after the training program [10].

In light of this information, the increasing population in our country and in the world and the increased responsibilities of pharmacists as pharmacy management and health professionals demonstrate the need for pharmacy technicians. In this regard, the workforce support of pharmacy technicians, who play a significant role in pharmacy management, is necessary for pharmacists to provide patient-centered healthcare services. At this point, working with pharmacy technicians who are appropriately trained and qualified in their respective fields allows the pharmacists to find more time as pharmacy managers and health professionals. In our country, the education of pharmacy technicians has undergone various changes over the years and has taken its current form. There is also a variety in the world definition of this sector; it varies from pharmacy technicians who ensure their professional adequacy with a comprehensive program, as in our country, to a situation that is not recognized or does not exist as a workforce. The point we will emphasize in this difference is the training curricula created by different institutions in many parts of the world, and the conditions required for pharmacy technicians to reach their competencies in their fields. Furthermore, it is also thought that the appropriate training of pharmacy support staff in their fields will increase their employment and support their need for training. At this point, Türkiye, the United States of America, the United Kingdom and France were selected as the four countries to focus on. The countries significantly differ from each other socially, culturally and economically. In addition, their main education systems are also significantly different from each other. It is considered that this situation will reveal the difference between the trainings more clearly.

2. MATERIALS AND METHODS

In March 2025, the countries to be included in the study were identified by searching key library databases using a comprehensive list of search terms. The various databases were searched using terms such as 'pharmacy technician' AND 'education' OR 'training'. Then, for each country, the organizations providing training for pharmacy technicians in that country were examined. In this way, the study is the result of an extensive literature review and organization research.

In order to be able to work as a pharmacy technician in Türkiye, as mentioned before, personnel must successfully graduate fromthe 2-year education of the Pharmacy Services program of the vocational schools affiliated to universities [2]. There are 112 institutions in Türkiye where there is a Pharmacy Services program and students must get the appropriate scores for the university conditions in order to receive education in these vocational schools and are placed in the university as a result

of the scores they receive [11]. In the Pharmacy Services Program, students are taught both theoretical and practical lessons, depending on the Vocational School they are affiliated with, such as Introduction to Pharmacology, Basic Anatomy, Basic Chemistry, Physiology, Medicinal Plant Products, Basic Microbiology lessons, Drug Information, Disease Information and Basic Pharmacy Practices in Pharmacy Services. The students are subjected to a midterm exam, midterm study (project, seminar, quiz, homework or a second midterm exam) and final exam for each course. The Midterm exam and midterm study, if available, contribute 40% to the success grade, while the final exam contributes 60%. The students must be successful in all the courses they have completed. In addition, the students must have at least 120 ECTS credits in the program and their GPA must meet the success requirements of the university they are affiliated with. The program also requires a 30-day internship. Students are entitled to graduate when they meet these conditions as a result of the education they receive for 2 years. Graduated students can be employed in the community or hospital pharmacies, pharmaceutical depots and pharmaceutical companies [12-14].

In the United States of America (USA), the education of pharmacy technicians has undergone a range of changes and has developed into what it is today. The Pharmacy Technician Accreditation Commission (PTAC), which was established to standardize and improve the quality of education and training programs for pharmacy technicians in the USA and to standardize education, published the Accreditation Standards for Pharmacy Technician Education and Training Programs in These requirements for accreditation were accepted by a protocol between the American Society of Health-System Pharmacists (ASHP) and the Accreditation Council for Pharmacy Education (ACPE) [15]. In addition to the accreditation conditions, a model curriculum was published for the institutions that will perform this certification, but it was stated that there is no obligation for the specified conditions, only an expectation in this direction [16]. In addition to these “entry-level” elements, pharmacy technicians who receive “advanced-level” training are expected to have a good knowledge of the drug research process, off-label drug use, chemotherapy drug preparations, and magistral drug manufacturing that does not require a high level of sterility [17]. The entry-level training program includes at least 400 hours of training over a minimum of 8 weeks, while the advanced training includes at least 15 weeks and 600 hours. These trainings consist of didactic, simulation and experiential training methods and the training hours are divided according to these training methods [17]. In January 2018, the Pharmacy Technician Certification Board (PTCB) announced several requirements for this training; candidates must have graduated from a pharmacy technician training program recognized by the PTCB or have at least 500 hours of work experience to be eligible to sit for the certification exam. Furthermore, the PTCB requires pharmacy technicians to complete at least 20 hours of training in pharmacy law and patient safety to renew their certification every 2 years. In addition, in-service training on various topics can provide them with another avenue for employment. A good example of this in-service training is the Compounding Sterile Preparation Technician (CSPT) training under the PTCB, an online training in sterile preparation for pharmacists and pharmacy technicians. Despite the breadth of training and opportunities, there is no obligation to attend training outside one state in the country and participation in pharmacy technician training is very low among pharmacy workers [18-20].

The training of pharmacy technicians in Great Britain was reviewed by the General Pharmaceutical Council (GPhC). And it was determined that there was no clear curriculum for pharmacy technician training in Great Britain. To work as a pharmacy technician, people must attend a GPhC-approved course and gain work experience under the direct supervision of a pharmacist or pharmacy technician for at least 14 hours per week for 2 years. They are then expected to pass a series of examinations demonstrating their competence against national standards. Although the training curriculum is not explicitly stated, pharmacy technicians are expected to have a basic knowledge of biology, chemistry, physiology, microbiology, and theoretical knowledge of topics such as pharmacy

science, pharmacy law, professional ethics, pharmacology, dispensing, patient communication, and pharmacy calculations. In addition, the GPhC has called for pharmacists and pharmacy technicians not to refrain from training and tutoring in setting training standards [21,22].

There are two different definitions in France: pharmacy technician and pharmacy assistant. Pharmacy assistants must first have worked in a pharmacy for two years to be eligible for certification. However, for pharmacy technicians, two years of pharmacy technician training is followed by a higher education diploma and the title of pharmacy technician. The pharmacy technician then undergoes one year of specialized training in a pharmacy or hospital and then receives the title of specialist pharmacy technician. This 2-year training for pharmacy technicians, which consists of an internship and theoretical courses, is usually provided by pharmacy faculties and pharmacy training centers. Furthermore, in order to graduate, the student must complete 120 ECTS credits and pass a continuous monitoring assessment. The curriculum is not sufficiently detailed, but the expectations of pharmacy technicians are similar to those in other countries [23,24].

3. RESULTS AND DISCUSSION

In this study, the training received by pharmacy technicians in Türkiye, England, the USA, and France was examined in detail. As a result of this examination, it was revealed that the training curriculum and expectations from pharmacy technicians were similar. The existence of these similarities creates a suitable area for standardizing the quality of education. On this point, the possibility of standardizing education will ensure that competent pharmacy technicians are trained in their fields and that their duties and responsibilities are also standardized. With the standardization of this education, pharmacy technicians will receive higher quality education and become more competent in their fields, which will increase the workforce support for pharmacists. This strategy also foresees that the pharmaceutical workforce, which is expected to be in short supply in the health system, will be strongly supported and the expected risk will be reduced. In addition to this standardization, the certification processes in the USA and the process of certificate renewal every two years and the lack of these conditions in our country, cause people to become monotonous and not to renew themselves in the profession. Creating an action plan by professional

Table 1. Comparison of different training conditions

	Türkiye	United States of America	Great Britain	France
Duration of Training	2 years	Entry-level- at least 8 weeks advanced-level- at least 15 weeks	2 years	2 years
Training organizations	Pharmacy Services Programs associated with Vocational Schools	Certification organizations	GPhC-approved courses	Faculty of Pharmacy, Pharmacy Education Centers
Conditions for Enrollment in the Training	To have a university entrance exam score suitable for the vocational school requirement	-	May vary based on the course provider. You may be expected to have the equivalent of four GCSEs at grade C or above, including math, English, science and one other subject.	Pharmacy assistants must have two years of professional activity in a pharmacy to be able to participate in a certification process.

boards and providing regular and compulsory in-service training will make a significant contribution to the competence of pharmacy technicians. Thus, it will improve the quality of support provided to pharmacists in pharmacy management.

3.1. Future Perspective for Pharmacy Technician Training and Workforce Support

By increasing the number of pharmacy technicians graduated from vocational schools in our country, the pharmacist's trust in the pharmacy technician who has received appropriate training will increase. This increase in trust will allow the role of pharmacy technicians to be expanded. The support of pharmacy technicians to pharmacists in pharmacy management will enable pharmacists to devote more time to patient-centered care services and provide adequate support to health institutions to further improve public health. However, the time allocated for pharmaceutical care provided to the patient will be sufficient and subsequently, the quality of pharmaceutical care will increase. Increasing the quality of pharmaceutical care will increase the patient's trust in the pharmacist and pharmacy. In addition, this will provide time for good pharmacy practices to provide the patient with the necessary incentives for rational drug use and patient education. Reduced health costs with the correct application of rational drug use will contribute greatly to the national economy. In order to achieve all these benefits and to realize this transformation, pharmacy boards, pharmacy professional associations, pharmacy technician professional associations, pharmacy universities and pharmacy services programs affiliated to vocational colleges should establish unity.

4. CONCLUSION

The curriculum and expectations of pharmacy technicians in Türkiye, England, the United States, and France are similar, but the practices and responsibilities of pharmacy technicians are different. It is a fact that the instructors who provide pharmacy technician training differ between countries. In the USA and England, the instructors are usually not pharmacists or pharmacy technicians, while in Türkiye, they are expert pharmacists or chemistry or biology experts who have completed a master's degree, and practical lessons are usually given by pharmacists. The fact that the definition and responsibilities of pharmacy technicians have not yet been standardized around the world causes their training to differ from each other.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization; B.T, Methodology, B.T; Software, B.T.; Validation, B.T.; Formal analysis, B.T.; Investigation, B.T. ; Resources, B.T. ; Data curation, B.T. ; Writing—original draft preparation, B.T. ; Writing—review and editing, B.T. ; Visualization, B.T. ; Supervision, B.T. ; Project administration, B.T. ; Funding acquisition, B.T. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declared that there is no conflict of interest.

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Review Article

Microwave-assisted synthesis of pyrazoles - a mini-review

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ABSTRACT

Heterocyclic compounds including pyrazole have a serious area in the field of medicinal chemistry and modern drug development studies. Pyrazole is a five-membered ring system containing two adjacent nitrogen atoms. Pyrazole attracts great attention due to its wide biological activity scale and potential in the development of new drug molecules. Traditional methods have been used in the general synthesis methods of pyrazole for a long time, but since these methods can be performed within certain limits, it is necessary to benefit from new and sustainable methodologies. In this review, the use and benefits of microwave (MW)-assisted techniques under the general title of green chemistry will be emphasized. The use of MW techniques stands out with its advantages such as increasing the efficiency of synthesis, obtaining selective products and preventing environmental pollution. The area of use of the MW method in pyrazole synthesis, synthesis mechanisms, organic synthesis methods and benefits are examined in this study. The MW method used in the synthesis of the pyrazole ring, which stands out with its bioactive diversity, will be encountered much more in the coming years.

Keywords: Pyrazole, Microwave, Green Chemistry

1. INTRODUCTION

Heterocyclic compounds, which constitute a large part of organic chemistry, also have a significant use in the field of pharmacy. More than 95% of the drugs and drug candidate molecules used today contain at least one heterocyclic ring [1]. Among these heterocyclic compounds, *N*-based compounds have been an important focus because they are found in the structure of many bioactive natural products such as antibiotics, vitamins, hormones and alkaloids used to improve human health and protect against diseases [2]. Therefore, *N*-based heterocyclic rings are the main focus in the synthesis and development of new drugs [3]. The five-membered pyrazole, which contains two adjacent nitrogens in its structure, is one of the heterocyclic compounds that draws organic and medicinal chemists' attention because of its strong biological activity and the variety that it can create in its chemical structure. It is also found in many drug molecules. Some examples are shown chronologically in Figure 1 and a few of them are shown in Figure 2. [4]. Pyrazole is synthesised through the reaction of a series of substituted hydrazine with an α,β-unsaturated carbonyl molecule, known as chalcones (Figure 3). In some instances, conventional organic synthesis techniques may prove insufficient for the synthesis of these compounds. New methodologies are currently being developed to overcome this limitation [5]. Among these methods, sustainable chemistry, also known as green chemistry, has become the focus [5]. The term 'green chemistry' was introduced in the mid-1990s

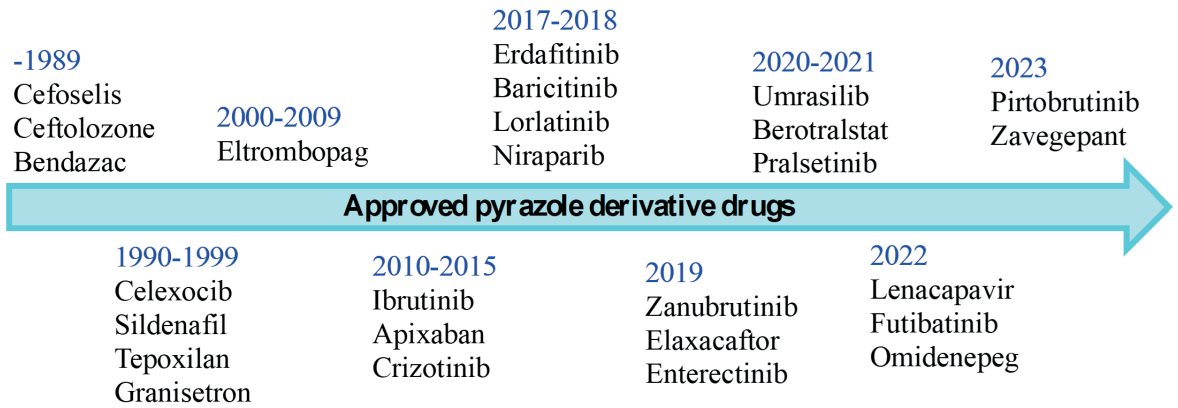


Figure 1. Chronological representation of some approved pyrazole derivative drugs [4,51].

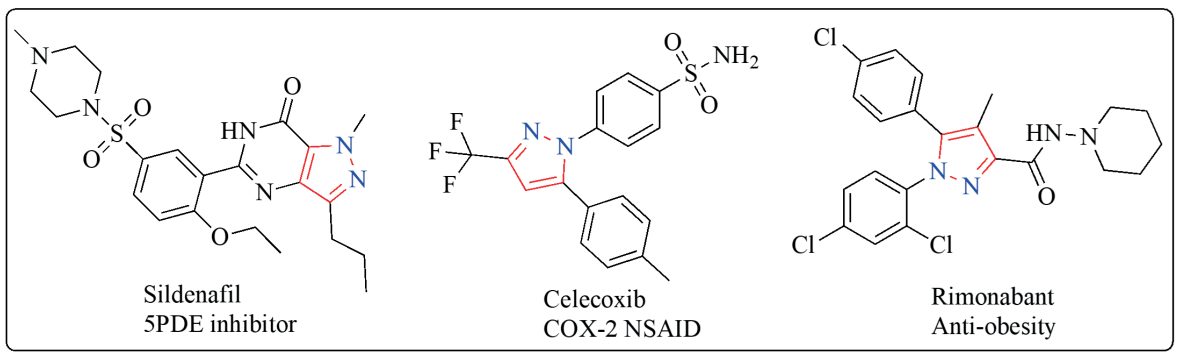


Figure 2. Examples of some biologically active pyrazole-containing drugs and their pharmacological effects [52].

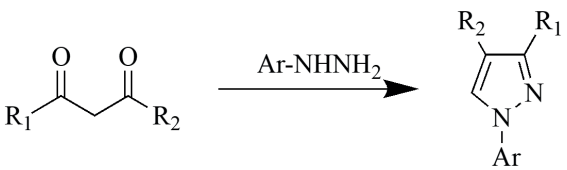


Figure 3. Synthesis of pyrazole from α,β-unsaturated carbonyl molecule [5].

for use in chemical syntheses to improve human health and protect the environment [6]. With green chemistry, the formation of hazardous compounds that may occur as a result of the use of traditional methods is reduced or even eliminated. The need for volatile organic solvents used in the synthesis of many heterocyclic compounds is also eliminated. Thus, sustainable, eco-friendly and clean new synthesis methods are created [6–8]. One of the most used green chemistry techniques in recent years is the microwave (MW) irradiation method [9]. Utilizing the MW technique in the synthesis of organic compounds accelerates the synthesis of compounds. It also offers superior targeting capabilities such as chemo-, regio-, and stereo-selectivity [10]. When past studies are examined, it is seen that MW has a wide range of applications. These can be listed as solvent-free reactions [11], heterocyclic chemistry [12], medicinal and combinatorial chemistry [13], homogeneous and heterogeneous catalysis [14], fullerene chemistry [15], cycloaddition reactions [16] and synthesis of radioisotopes [17]. Dielectric heating resulting from material–wave interactions is the cause of the acceleration rate seen in MW irradiation. MW is a representation of electromagnetic energy as it consists of an electric and magnetic field. This energy does not alter molecule structure, but it can function as a nonionizing radiation that rotates dipoles and generates ion molecular movements [18]. The MW field applied to the components allows the molecules it contains to spend a little more time in the electric field to which they are exposed, allowing the molecules to pass in the event of an arrangement, thus releasing thermal energy. In short, this method allows the absorption of MWs and their ability to withstand heat. Compared to traditional heating, this

interior warmth is substantially more homogeneous [19]. Method development in microwave-assisted organic synthesis is as follows: determination of open or closed application, solvent selection, determination of reaction conditions, adjustment of time and determination of exposure power, as shown in Figure 4 [20].

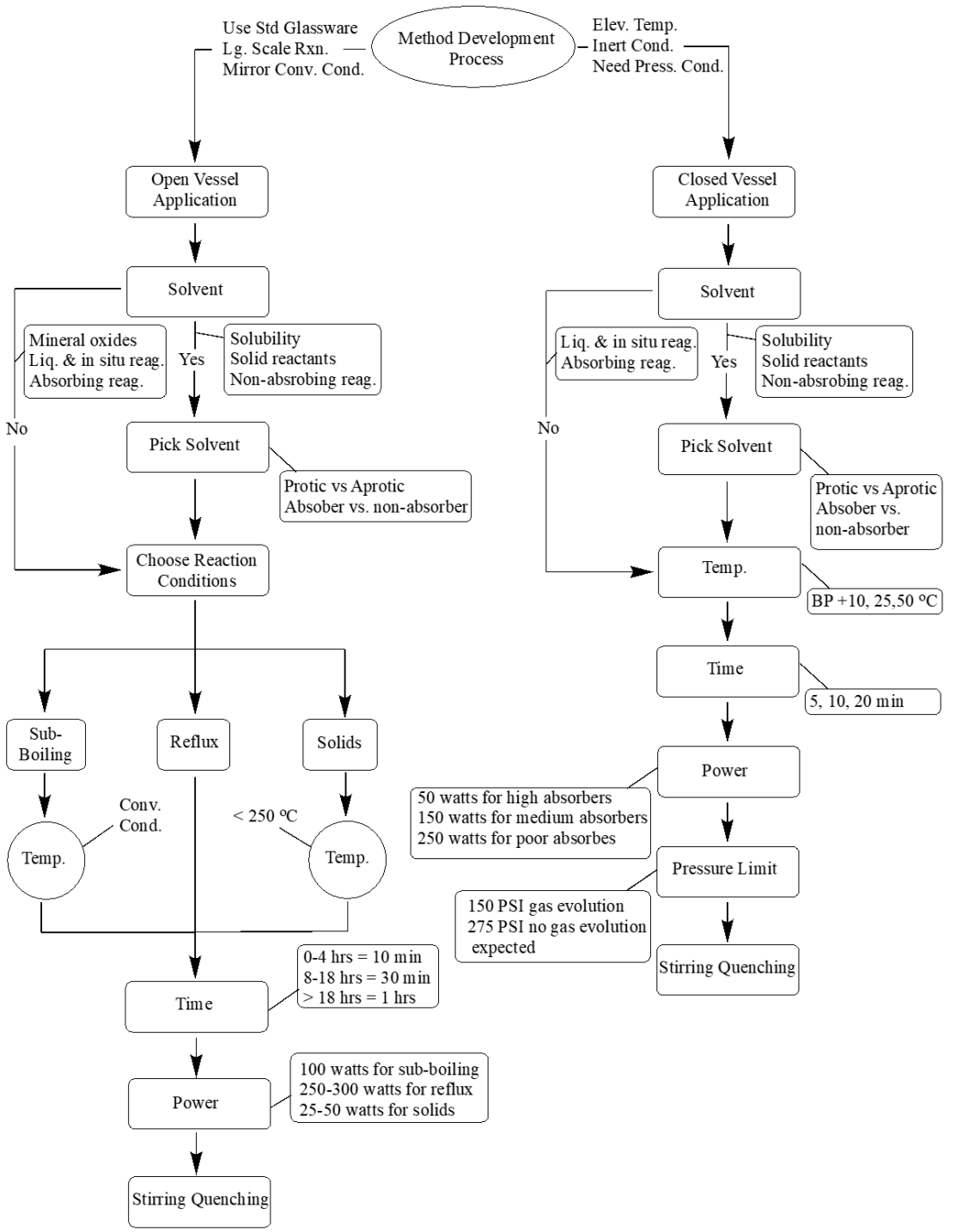


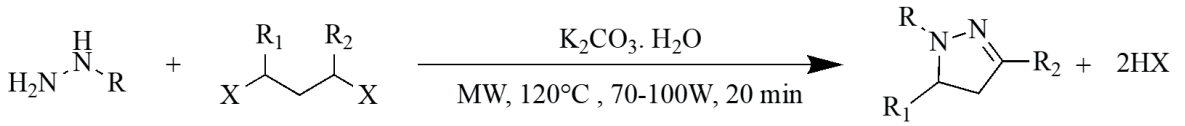
Figure 4. Microwave assisted synthesis method determination steps [20].

2. SYNTHESIS OF PYRAZOLE, USING MW METHOD

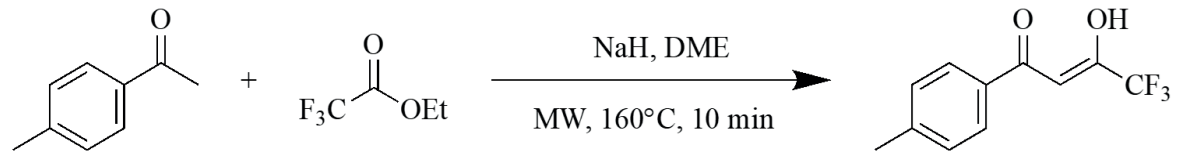
The azole family, which contains 5-membered heterocyclic nitrogenous rings such as pyrazole, imidazole, thiazoline and pyrazolidine, has a wide spectrum of biological activities, including antifungal [21], antiviral [22], anti-inflammatory [23], anti-tumor [24], anti-bacterial [25] and antioxidant [26]. This has caused them to become a focal point in the pharmaceutical industry [27]. With two nitrogen atoms positioned next to each other and a five-membered heterocyclic ring, pyrazole is a significant subgroup of the azole family. Because of their broad biological uses, these chemicals are the most investigated members of the azole family [28]. The first known pyrazole synthesis was described by Knorr [29]. Traditional pyrazole synthesis is not sufficient to obtain products with the desired yield. With the use of newly developed multimode and monomode microwave devices in the field of medicinal chemistry, products with higher yields can be obtained in a shorter time [30]. The results of recent studies indicate that both monomode and multimode microwave methods are reproducible [31,32]. This study will shed light on the MW-supported pyrazole syntheses made from past years to present.

In the study conducted by Ju et al. (2005), MW iridation method was used for pyrazole synthesis. In the method used, MW power was 70-100 W and the reaction took place in 20 minutes in aqueous medium in the presence of a weak base at 120°C. SN_2-like sequential heterocyclization reaction, which is difficult to perform by the conventional method, was carried out with a yield of 60-80%. (Scheme 1) [33].

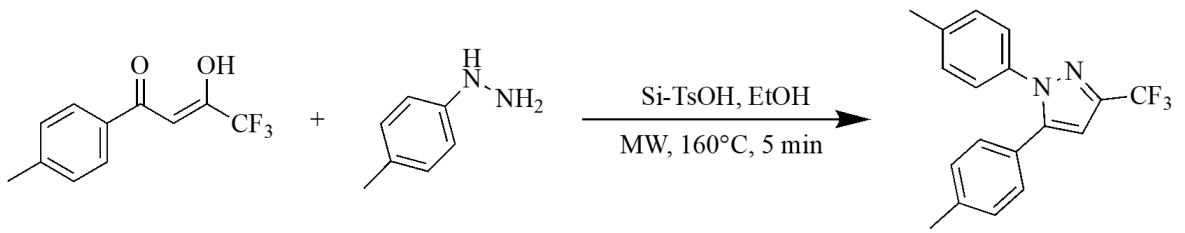
Paul et al. (2006) used the MW-assisted synthesis method for pyrazole synthesis. In the study, the first step of the two-step synthesis was completed in 5 days for the substances without MW application, while the MW-applied substances completed the reaction in a short time of 2 hours. In the first step, 4-methylacetophenone and ethyl trifluoroacetate were used at 160°C for 10 min and the desired enol ketone was obtained with 96% yield. In the second step, the most efficient conditions for the reaction between the obtained enol ketone and 4-methylphenylhydrazine are the method using silica-supported toluenesulfonic acid as a proton source at 160°C in the microwave (Scheme 2 and Scheme 3) [34].



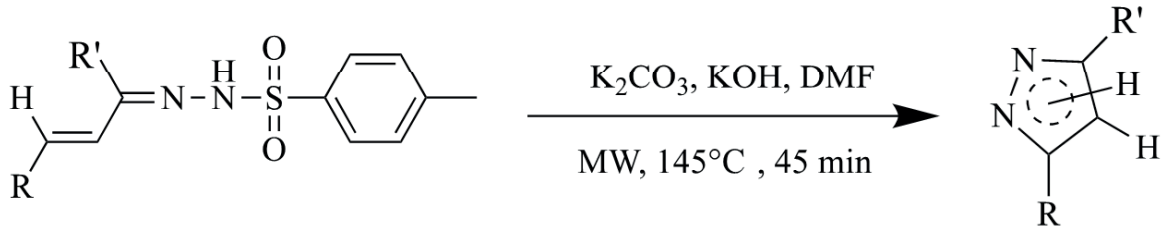
Scheme 1. Double alkylation of hydrazine by alkyl dihalides under MW irridation.



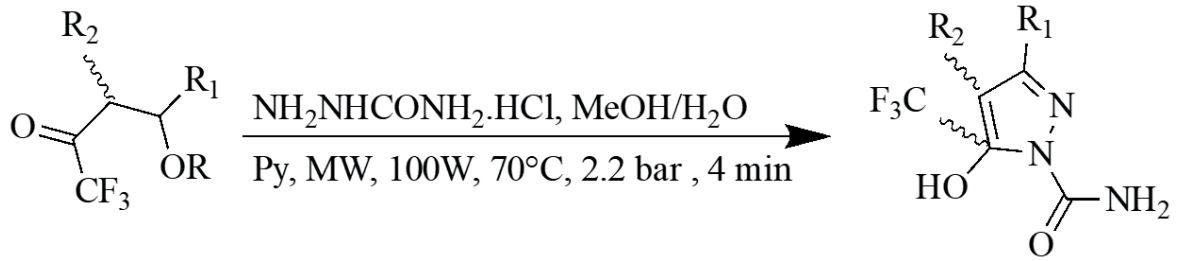
Scheme 2. Synthesis of (*Z*)-4,4,4-trifluoro-3-hydroxy-1-(*p*-tolyl)but-2-en-1-one.



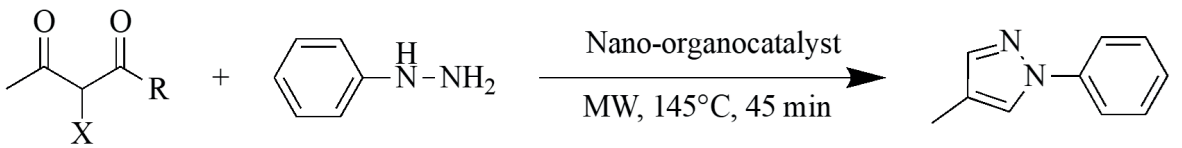
Scheme 3. Synthesis of 1,5-di-*p*-tolyl-3-(trifluoromethyl)-1*H*-pyrazole.



Scheme 4. Scheme of reaction for converting tosylhydrazones into pyrazoles.



Scheme 5. Synthesis of 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles.



Scheme 6. Synthesis of pyrazole derivatives using nano-organocatalyst by MW heating.

Corradi et al. (2007) synthesized new pyrazole derivatives by microwave method starting from acyclic α,β-unsaturated carbonyl compound. Synthesis is carried out by 1,3-cycloaddition of the corresponding tosylhydrazones of the selected carbonyl compounds using a base in dry medium (Scheme 4) [35].

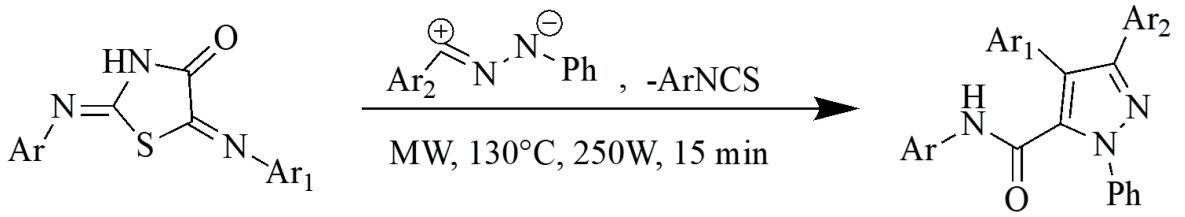
In the study conducted by Sauzem et al. (2008), a new pyrazole derivative compound was synthesised using MW in the second step of a two-step synthesis study. In the initial stage of the process, 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles were synthesised through a cyclocondensation reaction involving enones and semicarbazide hydrochloride. In the second step, a mixture of enones and semicarbazide hydrochloride was reacted in the presence of pyridine and a methanol/water solution (3:1 v/v) as solvent. The solution was subjected to microwave irradiation (100 W) at 70°C and 2.2 bar pressure for 4 minutes, resulting in the production of 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles with an 82-96% yield (Scheme 5) [36].

In the study conducted by Polshettiwar et al. (2009), MW-supported pyrazole derivatives were synthesized to be used as nano-organocatalysts in water. The conditions in this synthesis were as follows: 140°C, 20 minutes (Scheme 6) [37].

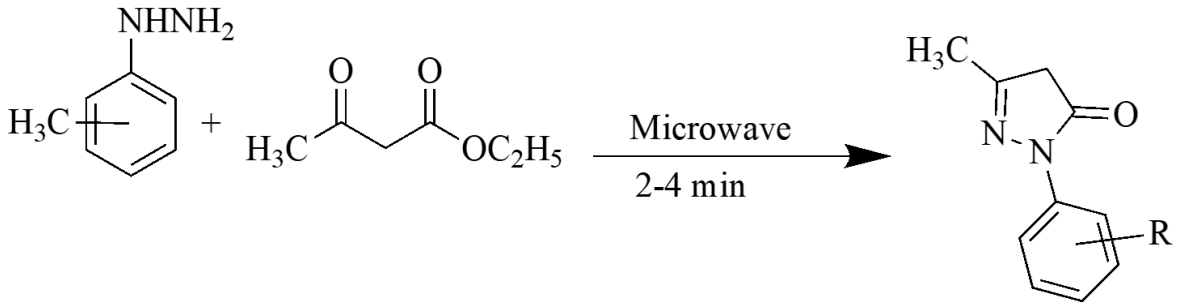
By 1,3-di-polar cycloaddition of nitrilimines with 5-arylidene-2-arylimino-4-thiazolidinones, Hatem et al. (2010) reported synthesizing 1,3,4-triaryl-5-*N*-arylpyrazole-carboxamides at 130°C for 15 minutes without the need of a solvent and with the assistance of MW (Scheme 7) [38].

In the study conducted by Antre et al. (2011), 3-methyl-1-substituted-1*H*-pyrazol-5(4*H*)-ones derivatives were obtained by reacting ethyl acetate with 1-phenylhydrazine under microwave for 2-4 minutes (Scheme 8) [39].

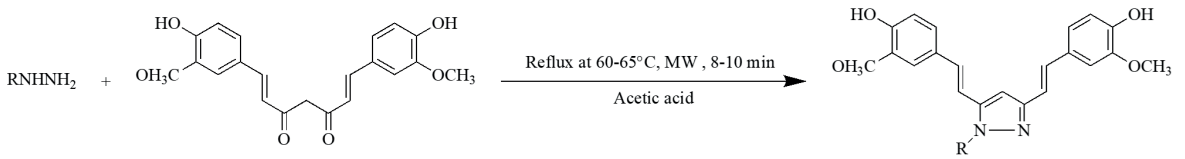
In a study conducted by Sahu et al. (2012), the objective was to obtain the derivatives of benzylidene of curcumin and new pyrazole derivatives using hydrazine. The initial synthesis was conducted



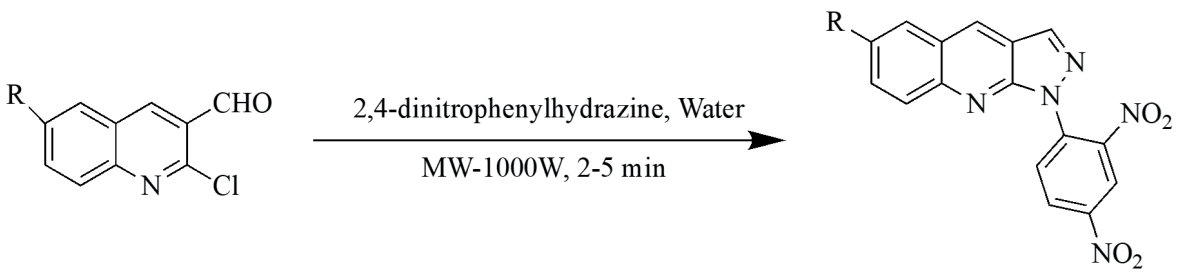
Scheme 7. Synthesis of 1,3,4-triaryl-5-*N*-arylpyrazole-carboxamides under MW irradiation.



Scheme 8. Synthesis of 3-methyl-1-substituted-1*H*-pyrazol-5(4*H*)-ones derivatives.



Scheme 9. Curcumin-pyrazole preparation.

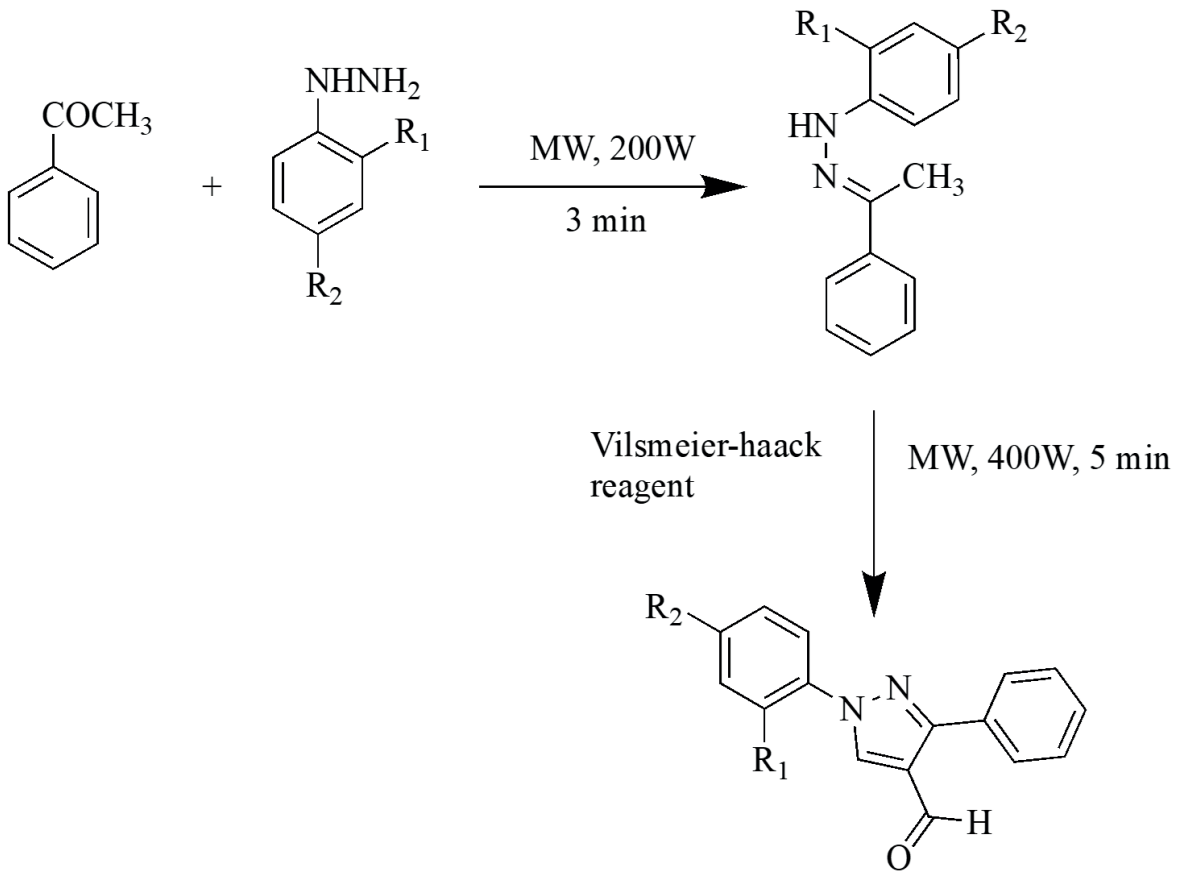


Scheme 10. Synthesis of 1-carboamidopyrazolo[3,4-b]quinolines derivatives.

via reflux in acetic acid at 60-65°C. However, due to the prolonged duration of the classic method (approximately 20 hours), a novel synthesis approach was devised. This involved performing the reaction in a microwave under non-solvent conditions, which proved to be an efficient and expeditious method, with the reaction completing in 8-10 minutes (Scheme 9) [40].

In a study conducted by Alam et al. (2013), a combination of conventional and unconventional methods was employed for the synthesis of 1-carboamidopyrazolo[3,4-b]quinolines. In the conventional synthesis method, it was refluxed in ethanol for a longer time than in the method utilising microwave irradiation. In the unconventional method, 2-chloroquinoline-3-carbaldehyde and 2,4-dinitrophenylhydrazine were refluxed with stirring in water under microwave irradiation at 1000W for 2-5 minutes (Scheme 10) [41].

Selvam T. P. et al. (2014) followed a two-step microwave-assisted synthesis method to synthesize new pyrazole derivatives. In the first step,

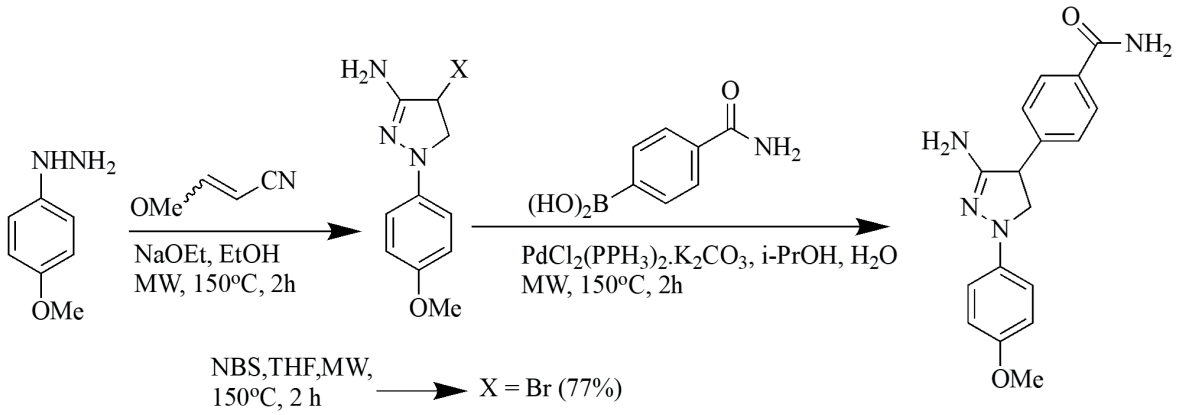


Scheme 11. Synthesis scheme of pyrazole-4-carbaldehyde derivatives.

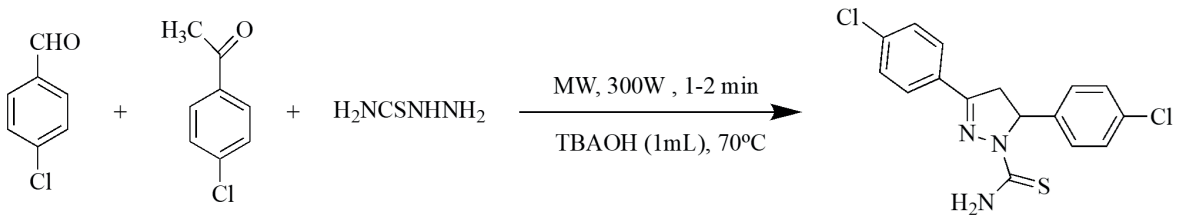
acetophenone, substituted phenylhydrazine and DMF were reacted under 200W microwave for 3 min to obtain 1-substituted phenyl-2-(1-phenylethyldiene) hydrazine. In the second step, 1-substituted phenyl-2-(1-phenylethyldiene)hydrazine was reacted with Vilsmeier-Haack reagent ($POCl_3$–DMF/SiO_2) under 400W microwave. It took a total of 5-6 min to obtain the final product (Scheme 11) [42].

In a study conducted by Bagley et al. (2015), the synthesis of novel pyrazole derivatives as MK2 inhibitors was pursued through the utilization of microwave-assisted Suzuki-Miyaura cross-coupling reactions. To this end, a microwave-assisted three-step synthesis method was developed. In the initial stage of the process, the 4-methoxyphenylhydrazine hydrochloride salt and 3-methoxyacrylonitrile were subjected to a reaction under alkaline conditions (using sodium ethoxide in ethanol) at 150°C for two hours in a microwave irradiation, resulting in the formation of 3-aminopyrazole. The subsequent step involved the synthesis of the pyrazolyl bromide compound, which was achieved by subjecting the reaction mixture to microwave irradiation at 150°C for 2 hours in the presence of NBS in THF, resulting in the formation of a C-4 brominated pyrazole with a 77% yield. To obtain the final pyrazole product, the Suzuki-Miyaura pyrazolyl bromide was combined with the 4-carbamoylphenylboronic acid in *i*PrOH–H2O in the presence of bis(triphenylphosphine) palladium(II) chloride and potassium carbonate under microwave irradiation at 150°C for 2 h, resulting in the formation of the complex pyrazole final product in 54% yield (Scheme 12) [43].

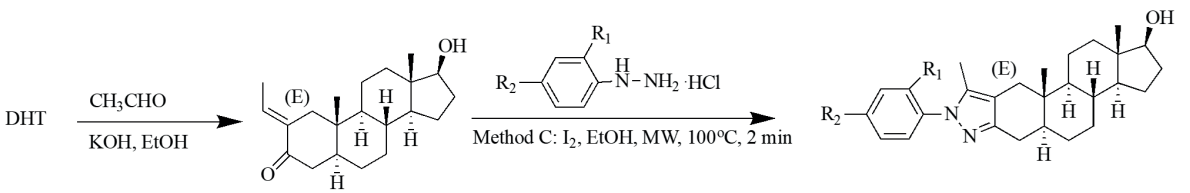
Farmani et al. (2018) devised and synthesised 4,5-dihydro-1*H*-pyrazole-1-carbothioamide in water using a microwave-assisted green chemistry method. In this study, a range of basic catalysts were employed, including NaOH, KOH, Et_3N and DABCO. However, the highest yield was observed in the derivative prepared using tetrabutylammonium hydroxide



Scheme 12. Microwave-assisted synthesis of the complex pyrazoles using a Suzuki-Miyaura cross-coupling.



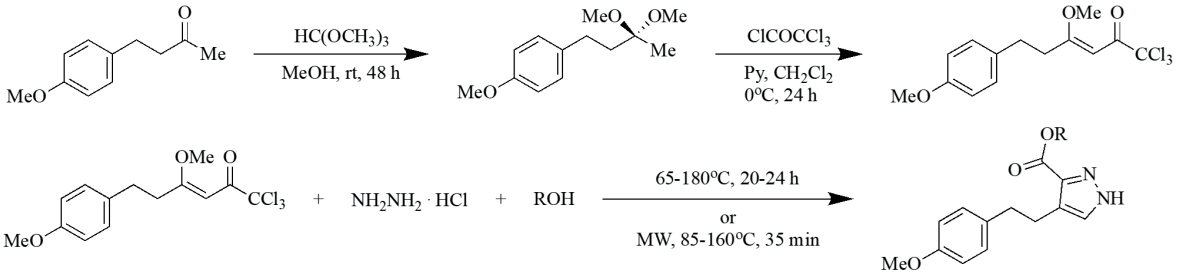
Scheme 13. Synthesis of 4,5-dihydro-1*H*-pyrazole-1-carbothioamides in the presence of TBAOH under microwave irradiation.



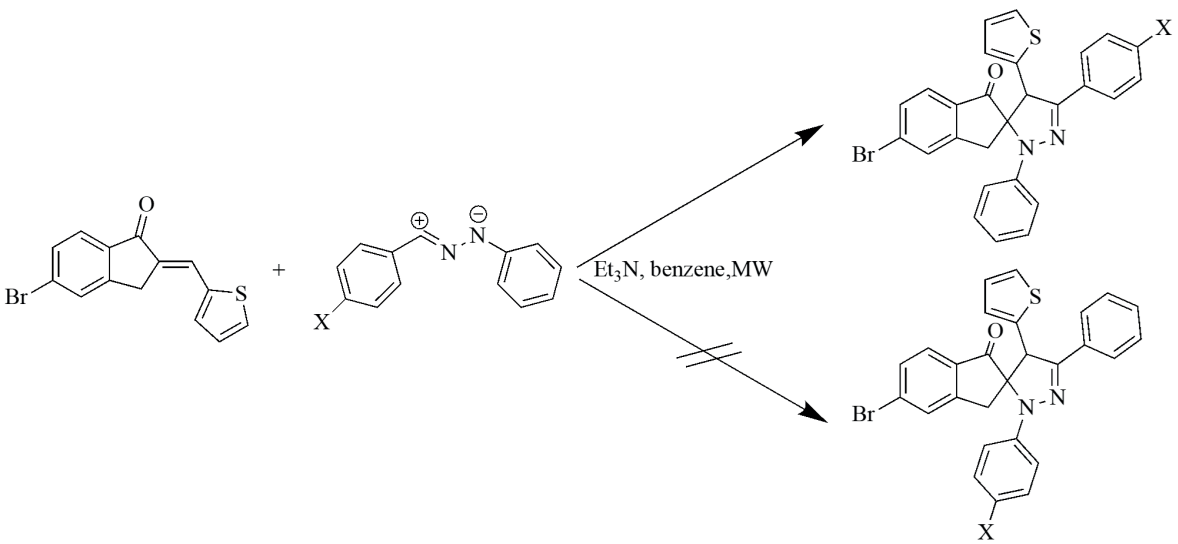
Scheme 14. Synthesis of ring arylpyrazole derivatives of DHT.

(TBAOH). Similarly, a variety of microwave irradiations were attempted, with the highest yield obtained at 300 W. The optimal temperature for the reaction was determined to be 70°C. The reaction of 4-chloro-benzaldehyde, 4-chloroacetophenone and thiosemicarbazide under the aforementioned conditions yielded pyrazole derivative compounds (Scheme 13) [44].

Mótyán et al. (2019) employed a microwave-assisted synthesis method for the design and synthesis of novel arylpyrazol derivatives as 17-keto analogues. In this study, in order to obtain the α,β-enone suitable for heterocyclization required in the pyrazole synthesis, DHT was first subjected to aldol condensation with an excess of acetaldehyde in alkaline ethanol at low temperature. Three distinct methodologies were employed: Method A, Method B, and Method C. While Method A encompasses the synthesis of compounds through conventional techniques, Methods B and C involve the utilisation of microwave-assisted synthesis. Method B was completed in a significantly shorter timeframe compared to Method A; however, the yield did not meet the desired specifications. Method C employs a one-pot procedure that includes I_2-mediated oxidative cyclisation and microwave irradiation. This approach was adopted to circumvent the necessity for a multi-step synthesis and to preclude the formation of unstable pyrazole by-products. In the last step of the synthesis, Jones reagent in acetone was used for the oxidation of the 17-OH group. The yield of the final products was found to be in the range of 81-89% (Scheme 14) [45].



Scheme 15. Synthesis of 1*H*-pyrazole-5(3)-carboxylates derivatives.

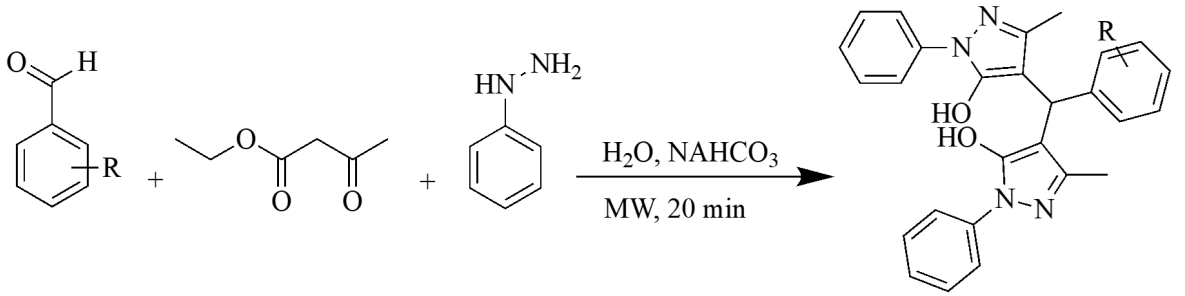


Scheme 16. Synthesis of spiropyrazoles under microwaves irradiation.

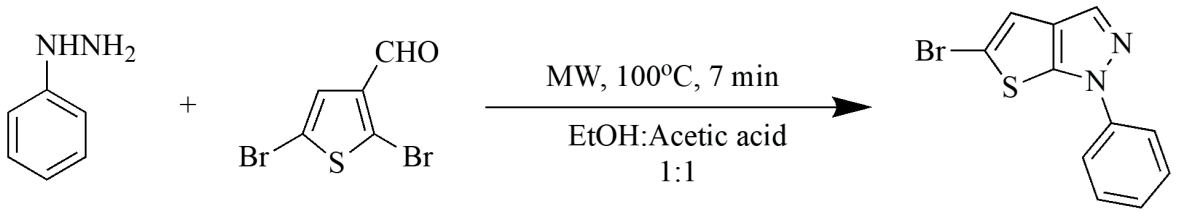
Goulart et al. (2020) synthesized 1*H*-pyrazolecarboxylates by both conventional and microwave-assisted synthesis methods, starting from compounds with antioxidant activity. The desired product was obtained as a result of a three-step synthesis. In the first step, trimethyl orthoformate and 4-(4-methoxyphenyl)butan-2-one were reacted in methanol at room temperature for 48 hours. The resulting product is 1-(3,3-dimethoxybutyl)-4-methoxybenzene. In the second step, benzene derivatives were acetylated with trichloroacetyl to form the precursor compounds required for the synthesis of the target compounds. In the last step, the reaction between $NH_2NH_2 \cdot HCl$ and 1,1,1-trichloro-4-methoxy-6-(4-methoxy-phenyl)hex-3-en-2-one was carried out using both microwave-assisted and conventional techniques. When this reaction was compared, it was observed that the microwave-assisted synthesis was significantly faster. (Scheme 15) [46].

In a study conducted by Masaret (2022), new spiropyrazole derivatives were synthesized using microwave irradiation and their antimicrobial, antiviral activities and effects on the novel coronavirus disease (2019-nCoV) were investigated. In the synthesis stages, the reaction between 5-bromo-2-thiophen-2-ylmethylene-indan-1-one and hydrazonoyl chloride was carried out under the following conditions: the reaction was carried out in benzene under microwave irradiation. In addition to the shortening of the reaction time, when the spectral data of the obtained products were examined, it was observed that substituted derivative products of the 4th position of the phenyl ring at the 5th position of the compound were obtained (Scheme 16) [47].

Anwer et al. (2023) synthesized novel pyrazole derivative compounds that can be used in cancer treatment using green chemistry methodologies. The desired pyrazole derivative compounds were



Scheme 17. Synthesis of bis-pyrazole analogues under MW irridation.



Scheme 18. Synthesis of 5-bromo-1-phenyl-1*H*-thieno[2,3-*c*]pyrazole compound.

obtained by reacting the mixture of enaminonitrile and malononitrile with cyanoacetamide or chloroacetic acid in the presence of sodium ethoxide under microwave radiation. The microwave used in the synthesis is 300 MW and the reaction time is 2-4 minutes [48].

Novel pyrazole derivative compounds based on substituted benzaldehyde were synthesized by Kumar et al. (2024). In this study, a novel pyrazole derivative compound was synthesized by subjecting the mixture of substituted benzaldehyde, ethyl-3-oxobutanoate, phenylhydrazine and water to microwave irradiation at room temperature for 20 min (Scheme 17) [49].

In a recent study, Sharma et al. (2024) employed a microwave-assisted synthesis approach to create novel pyrazole derivatives, beginning with a carbaldehyde derivative compound. The synthesis was conducted using a one-pot microwave irradiation method. A reaction was conducted between phenyl hydrazine and 2,5-dibromo-3-thiophenecarbaldehyde in a solution of ethanol and acetic acid in a 1:1 ratio. The temperature was maintained at 100°C, and the reaction was irradiated in a microwave oven for a period of seven minutes (Scheme 18) [50].

3. CONCLUSION

Many drugs and drug candidates that have been the subject of medicinal chemistry contain heterocyclic rings. Among these rings, pyrazole, an N-based heterocyclic ring, stands out for its diversity of biological activities and ease of access. Until recent years, pyrazole synthesis studies were carried out using classical methods, but with the emergence of the term green chemistry and the widespread use of MW irridation, new environmentally friendly, faster and higher yielding methods have begun to be applied. In this review, studies including MW synthesis methods used for pyrazole synthesis have been examined. The use of microwave radiation is also important in an industrial sense, as it keeps the environment clean and provides clean products. It is obvious that it will be used more frequently in the field of chemistry and medicine in the coming years. Considering the general review, for microwave-assisted pyrazole synthesis, microwave irradiation should be in the range of 100-300 MW, temperature should be in the range of 65-180 °C, and time should be in the range of 5-45 minutes. A comparison of the study conducted by Sahu et al. (2012) with that conducted by Selvam et al. (2014) reveals that the utilisation of solvent in the reaction does not impact reaction time. However, it has been demonstrated that solvent-free reactions

can contribute to the prevention of environmental pollution, less hazardous reaction conditions and reduced consumption, thus being compatible with the principles of 'green chemistry'. Among the studies examined, the conditions that achieved the highest efficiency (82-96%) were as follows: (100 W) at 70°C and 2.2 bar pressure for 4 minutes.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization, A.K.; Methodology, A.K.; Software, A.K.; Validation, A.K.; Formal analysis, A.K.; Investigation, A.K.; Resources, A.K.; Data curation, A.K.; Writing—original draft preparation, A.K.; Writing—review and editing, A.K.; Visualization, A.K.; Supervision, A.K.; Project administration, A.K.; Funding acquisition, A.K. The author have read and agreed to the published version of the manuscript.

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Conflict of interest

The author declared that there is no conflict of interest.

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