Short Communication:

Ultrasound-Assisted One-Pot Synthesis and Anti-Inflammatory Evaluation of 2,4,5-Trisubstituted Imidazoles with Hemocompatibility Assessment

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Abstract: *Imidazole derivatives are well known for their anti-inflammatory potential, and recent studies focus on synthesizing 2,4,5-trisubstituted imidazoles through faster, greener methods. In the present study, a series of such derivatives (***N1–N10***) was prepared in a single-pot, ultrasound-assisted reaction using benzil, various aromatic aldehydes, and ammonium acetate, with sulfuric acid as the catalyst. This procedure shortens reaction times, enhances yields, and reduces waste, offering a greener alternative to conventional heating methods. The products were fully characterized by ^{1}H-NMR, ^{13}C-NMR, FTIR, and MS. Four representative derivatives (***N2, N4, N5,*** *and* **N8***) were selected for biological evaluation. Their anti-inflammatory activity was assessed* in vitro *using trypsin inhibition and albumin denaturation assays, with diclofenac sodium as the reference.* **N5** *and* **N8** *showed potent trypsin inhibition (61.63 ± 1.74 and 53.49 ± 0.45%) comparable to diclofenac (61.69 ± 0.74%), while* **N2** *achieved 60.71 ± 3.68% and* **N4** *50.33 ± 0.58%. In albumin denaturation,* **N5** *and* **N8** *again displayed high activity (76.8 ± 6.3 and 76.55 ± 0.89%),* **N2** *58.33 ± 0.62% and* **N4** *50.33 ± 0.58%. All compounds produced < 5% hemolysis, indicating good hemocompatibility. The findings demonstrate that ultrasound enables a greener and more efficient synthesis of anti-inflammatory imidazole derivatives, while also highlighting these compounds as promising, safe, and effective candidates for future therapeutic development.*

Keywords: *synthesis; 2,4,5-trisubstituted imidazoles; anti-inflammatory; ultrasound; hemocompatibility*

■ INTRODUCTION

Imidazole derivatives represent a significant class of nitrogen-containing heterocycles that have attracted considerable attention in medicinal chemistry due to their structural diversity, synthetic accessibility, and extensive pharmacological potential [1-2]. Among these, 2,4,5-trisubstituted imidazole derivatives are particularly noteworthy for their capacity to interact with various biological targets, making them valuable scaffolds in the design and development of antifungal [3], antimicrobial [4], antibacterial [5], anticancer [6], and anti-inflammatory agents [7]. Chronic inflammation is a hallmark of many cardiovascular, autoimmune, and neurodegenerative disorders [8]. It involves the continuous activation of immune pathways, the release of cytokines, and the induction of oxidative stress, leading to progressive tissue damage [9-10]. Imidazole derivatives are increasingly investigated for their anti-inflammatory effects, particularly their modulatory roles on biochemical mediators such as prostaglandins and histamine [11-13]. Several studies have reported that structural modifications to the imidazole nucleus significantly influence their interaction with inflammatory pathways [14-16].

Significant progress has been made in recent decades in the synthesis of 2,4,5-trisubstituted imidazoles. Modern synthetic methods, including multicomponent reactions, are used to improve pharmacokinetic and pharmacodynamic profiles [17-18]. In alignment with green chemistry principles, several environmentally benign Brønsted acid catalysts have been employed to synthesize 2,4,5-trisubstituted imidazoles. These include conventional sulfuric acid [18], protic ionic liquids [19], boric acid [20], and acidic ionic liquids [21]. Additionally, sulfonic acid-functionalized catalysts such as pumice@SO_3H have been explored for their biodegradability, reusability, and catalytic efficiency in multicomponent imidazole synthesis [22]. Likewise, silica-organic and rice husk-derived Brønsted acid catalysts provide reusable and efficient alternatives for preparing 2,4,5-trisubstituted imidazoles [23-24].

This study focuses on the green synthesis of 2,4,5-trisubstituted imidazole derivatives through a one-pot, ultrasound-assisted multicomponent reaction involving 1,2-diketones, aromatic aldehydes, and ammonium acetate. The ultrasound methodology improves reaction efficiency, reduces reaction time, and aligns with sustainable chemistry practices. The synthesized compounds were evaluated *in vitro* for their anti-inflammatory properties using albumin denaturation and trypsin inhibition assays, with diclofenac sodium as the reference drug. Additionally, hemolytic activity was assessed to determine biocompatibility. These evaluations underscore the therapeutic promise and membrane safety of the developed imidazole derivatives. Despite advances in synthesizing 2,4,5-trisubstituted imidazoles, most methods still rely on traditional heating and long reaction times, with limited studies on anti-inflammatory effects or hemocompatibility. Although ultrasound-assisted techniques are documented, no research has integrated this eco-friendly process with dual biological assessments of both anti-inflammatory efficacy and hemocompatibility.

■ EXPERIMENTAL SECTION

Materials

Chemicals such as benzil (≥ 99%), ammonium acetate (≥ 98%), bovine serum albumin (BSA, ≥ 98%), and aromatic aldehydes with purities ranging from 95 to 99% were procured from Sigma-Aldrich. Concentrated sulfuric acid (98%) and absolute ethanol were sourced from Fluka. Trypsin (from porcine pancreas, ≥ 10,000 BAEE units/mg protein) was obtained from an Indian manufacturer. Tris buffer and phosphate-buffered saline (PBS, pH 7.4) were obtained from Himedia (India) and used according to the manufacturer's instructions. Analytical-grade trichloroacetic acid (TCA, ≥ 99%) was utilized as received. Diclofenac sodium (100 mg tablets, from a commercial source) was purchased from a local pharmacy and served as the reference anti-inflammatory agent. All chemicals and solvents used were of analytical grade and were employed without further purification.

Instrumentation

An SS-6820C ultrasonic cleaner operating at 40 kHz was employed for ultrasonic irradiation. The melting points of the synthesized compounds were determined using a microprocessor-based melting point apparatus and open capillary tubes. Thin-layer chromatography (TLC) using Merck 60GF254 aluminium-backed silica plates was employed to monitor the progress of the reactions. Fourier-transform infrared (FTIR) spectroscopy was conducted using potassium bromide disks on a SHIMADZU FTIR-8400S spectrophotometer. ^1H-NMR spectra were acquired with an INOVA (500 MHz) NMR spectrometer, employing DMSO-d_6 as the solvent and tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were obtained using an Agilent Technology (HP) 5973 system. UV-vis spectra were recorded on a UV-1800 spectrophotometer.

Procedure

*Synthesis of 2,4,5-tri-imidazole derivatives (***N1–N10***)*

Conventional methods. A mixture of benzil (1 mmol, 0.2102 g), aldehyde (1 mmol; quantities listed in Table S1), and ammonium acetate (3 mmol, 0.3854 g) as ammonia source was prepared in 10 mL of ethanol, with three drops of sulfuric acid acting as a catalyst. The progress of the reaction was monitored using TLC with a 3:2 mixture of *n*-hexane and ethyl acetate as the eluent. After completion, the reaction mixture was poured into 50 mL of cold water, forming a precipitate. This precipitate was filtered, washed with water, dried, and recrystallized from hot ethanol to yield the desired 2,4,5-trisubstituted imidazole derivatives.

Ultrasound methods. A mixture containing benzil (1 mmol, 0.2102 g), an aldehyde (1 mmol, Table S1), and ammonium acetate (3 mmol, 0.3854 g) as the nitrogen donor was dissolved in 5 mL of ethanol. Three drops of concentrated sulfuric acid were then added as a catalyst. The solution was subjected to ultrasonic irradiation at 40 °C for an appropriate duration. The reaction progress was tracked via TLC utilizing a 3:2 mixture of *n*-hexane and ethyl acetate as the eluent. After filtering the reaction mixture, the solid was washed with distilled water, dried, and dissolved in hot ethanol to produce the imidazole derivatives (***N1–N10***).

Biological evaluation

To determine the anti-inflammatory effects of 2,4,5-trisubstituted imidazole derivatives, selected compounds (***N2***, ***N4***, ***N5***, and ***N8***) were screened for their *in vitro* activity by evaluating protein inhibitory action and the inhibition of albumin thermal denaturation. Diclofenac sodium was used as a reference.

Protein inhibition assay

Serial dilutions of the synthesized 2,4,5-trisubstituted imidazole derivatives and diclofenac sodium were prepared at concentrations of 100, 250, 500, and 1000 µg/mL. For each test, 2 mL of the reaction solution was prepared by combining 1 mL of a 20 mM Tris-HCl buffer (pH 7.4) with 1 mL of the sample. The resulting mixture containing 0.01 mg of trypsin was incubated at 37 °C for 5 min. Subsequently, 1 mL of casein (0.8% w/v) was added to the mixture and incubated for 20 min. Then, 2 mL of 70% trichloroacetic acid was added to terminate the reaction. The reaction was halted, and the cloudy suspension was centrifuged. The absorbance of the supernatant was measured, and then the %inhibition was measured using Eq. (1);

$$\%\text{Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100\% \quad (1)$$

where A_{control} is the absorbance of the solution without any test sample, A_{sample} is the absorbance of the solution with 2,4,5-trisubstituted imidazole compounds and diclofenac sodium as a reference.

Albumin denaturation assay

Dilutions of each imidazole compound and diclofenac sodium were prepared at various concentrations (100, 250, 500, and 1000 µg/mL). To create the reaction mixture (0.5 mL), 0.45 mL of a 1% aqueous solution of bovine serum albumin was added to 0.05 mL of the test sample, which included the 2,4,5-trisubstituted imidazole compounds and diclofenac sodium. A small amount of HCl was added to adjust the mixture's pH to 6.3. All samples were incubated at 37 °C for 20 min and then heated to 55 °C for an additional 20 min. After cooling, the turbidity of the samples was assessed spectrophotometrically at 660 nm. The percentage inhibition of albumin denaturation was calculated using Eq. (1).

Hemolytic activity

This study involved ten healthy, non-smoking participants: six males and four females, aged between 18 and 30 years. Blood samples were obtained through venipuncture and placed in tubes with heparin as the anticoagulant. Different concentrations (1000, 500, 250, and 100 µg/mL) of synthesized compounds (***N2***, ***N4***, ***N5***, and ***N8***) were introduced into 30 µL to 0.2 mL of blood. The resulting mixture was gently stirred for 10 s, after which 10 mL of normal saline (0.9%) was added to reduce hemolysis. The samples were centrifuged at 4000×g for 10 min. To assess hemoglobin release, the absorbance of the supernatant was recorded at 540 nm. For total hemolysis (100%), blood was diluted 100-fold in distilled water. Normal saline served as the negative control, while Triton X-100 functioned as the positive control. The %hemolysis was calculated using Eq. (2);

$$\%\text{Hemolysis} = \frac{(A_T - A_S)}{A_{100\%} - A_S} \times 100\% \quad (2)$$

where A_T: Absorbance of test solution, A_S: Absorbance of normal saline, and $A_{100\%}$: Absorbance of 100% hemolysis

*Spectral and physical data for 2,4,5-trisubstituted imidazole derivatives (*N1–N10*)*

4-(4,5-Diphenyl-1*H*-imidazol-2-yl)benzonitrile *N1*. M.p. 246–250 °C (lit. 248–250 °C, [25]); FTIR (KBr, cm^{-1}): 3290 (N–H), 3055 (Ar–H), 2225 (C≡N), 1604 (C=N), 1492, 1438 (C=C); ^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ (ppm): 13.01 (*s*, 1H, NH), 8.27 (*d*, *J* = 6.4 Hz, 2H, ArH), 7.92 (*d*, *J* = 6.4 Hz, 2H, ArH), 7.62–7.24 (*m*, 10H, ArH); ^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ (ppm): 143.67, 138.09, 134.74, 134.26, 132.66, 129.52, 129.40, 128.63, 128.47, 128.17, 128.04, 127.10, 126.72, 125.49, 118.83, 110.08; MS (ESI): *m/z* = 321.4 [M^+].

4-(4,5-Diphenyl-1*H*-imidazol-2-yl)-2-methoxyphenol *N2*. M.p. 199–200 °C (lit. 198–200 °C, [26]); FTIR (KBr, cm^{-1}): 3514 (O–H), 3205 (N–H), 3059 (Ar–H), 2943 (C–H), 1674 (C=N), 1600 (C=C), 1276 (C–O), 1030 (O–CH_3); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 10.19 (*s*, 1H, NH), 9.41 (*s*, 1H, OH), 7.93 (*d*, *J* = 5.7 Hz, 1H, ArH), 7.68 (*d*, *J* = 1.5 Hz, 1H, ArH), 7.58 (*dd*, *J* = 6.6, 1.6 Hz, 2H, ArH), 7.53 (*d*, *J* = 5.7 Hz, 2H, ArH), 7.38 (*t*, *J* = 6.0 Hz, 4H, ArH), 7.32 (*d*, *J* = 5.9 Hz, 2H, ArH), 6.91 (*d*, *J* = 6.6 Hz, 1H, ArH), 3.88 (*s*, 3H, O–CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 147.72, 147.58, 145.75, 135.42, 132.39, 132.23, 129.50, 129.41, 128.39, 127.81, 127.27, 120.67, 118.78, 115.66, 109.69, 55.74; MS (ESI): *m/z* = 343. [M^{+1}].

4-(4,5-Diphenyl-1*H*-imidazol-2-yl)-*N*,*N*-dimethylaniline *N3*. M.p. 257–258 °C (lit. 256–257 °C, [27]); FTIR (KBr, cm^{-1}): 3479, 3221 (N–H), 3066 (Ar–H), 1635 (C=N), 1589 (C=C), 1303 (C–N). ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 9.68 (*s*, 1H, NH), 7.94 (*d*, *J* = 7.1 Hz, 2H, ArH), 7.52 (*t*, *J* = 6.3 Hz, 2H, ArH), 7.40–7.30 (*m*, 6H, ArH), 6.83 (*d*, *J* = 8.5 Hz, 2H, ArH), 2.98 (*s*, 6H, $N(CH_3)_2$); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 150.62, 146.10, 135.42, 132.25, 129.49, 129.41, 128.35, 127.78, 127.22, 126.64, 116.46, 111.79, 40.02; MS (ESI): *m/z* = 339.4 [M^{+1}].

4,5-Diphenyl-2-(thiophen-2-yl)-1*H*-imidazole *N4*. M.p. 257-260 °C (lit. 258-260 °C, [28]); FTIR (KBr, cm^{-1}): 3317 (N–H), 3066 (Ar–H), 1666 (C=N), 1594 (C=C), 686, 636 (C–S); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 12.82 (*s*, 1H, NH), 8.11 (*d*, *J* = 6.2 Hz, 1H, ArH), 7.55 (*d*, *J* = 7.7 Hz, 1H, ArH), 7.50–7.36 (*m*, 10H, ArH), 7.30 (*t*, *J* = 7.4 Hz, 1H, ArH); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 145.41, 135.25, 132.92, 130.10, 129.09, 128.90, 128.59, 128.47, 128.32, 128.25, 127.73, 127.59, 127.11, 125.21; MS (ESI): *m/z* = 303 [M^{+1}].

2-(3-Methylthiophen-2-yl)-4,5-diphenyl-1*H*-imidazole *N5*. M.p. 232–234 °C; FTIR (KBr, cm^{-1}): 3317 (N–H), 3063 (Ar–H), 2924, 2858 (C–H), 1666 (C=N), 1585 (C=C), 686, 632 (C–S); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 12.47 (*s*, 1H, NH), 7.58–7.53 (*m*, 6H, ArH), 7.52–7.36 (*m*, 4H, ArH), 7.23–7.00 (*m*, 1H, S-C–CH_3), 2.53 (*s*, 3H, CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 141.59, 141.18, 137.57, 135.72, 134.05, 131.09, 129.40, 129.27, 129.14, 128.64, 128.29, 127.61, 127.32, 126.86, 126.33, 15.07; MS (ESI): *m/z* = 316.4 [M^+].

2-(5-Methylthiophen-2-yl)-4,5-diphenyl-1*H*-imidazole *N6*. M.p. 226–227 °C (lit. 226 °C, [29]); FTIR (KBr, cm^{-1}): 3317 (N–H), 3066 (Ar–H), 1666 (C=N), 1589 (C=C), 682, 640 (C–S); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 12.69 (*s*, 1H, NH), 7.95 (*d*, *J* = 6.7 Hz, 2H, ArH), 7.84–7.68 (*m*, 1H, ArH), 7.66 (*t*, *J* = 6.3 Hz, 2H, ArH), 7.52–7.49 (*m*, 2H, ArH), 7.49–7.24 (*m*, 4H, ArH), 6.85 (*d*, *J* = 2.8 Hz, 1H, ArH), 2.50 (*s*, 3H, CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 141.60, 139.60, 135.42, 132.20, 131.50, 129.49, 129.41, 129.12, 128.65, 128.17, 128.14, 128.11, 127.61, 126.08, 14.89; MS (ESI): *m/z* = 316.1 [M^+].

2-(4-Methoxyphenyl)-4,5-diphenyl-1*H*-imidazole *N7*. M.p. 230–232 °C (lit. 230–233 °C, [30]); FTIR (KBr, cm^{-1}): 3437 (N–H), 3066 (Ar–H), 2870 (C–H), 1666 (C=N), 1589 (C=C), 1211 (C–O); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 10.07 (*s*, 1H, NH), 8.09 (*d*, *J* = 1.8 Hz, 2H, ArH), 7.54 (*d*, *J* = 5.6 Hz, 4H, ArH), 7.43 (*t*, *J* = 5.8 Hz, 4H, ArH), 7.38 (t, *J* = 5.8 Hz, 2H, ArH), 7.15 (*d*, *J* = 7.0 Hz, 2H, ArH), 3.85 (*s*, 3H, OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 160.77, 144.73, 130.38, 130.15, 129.48, 129.41, 128.59, 128.44, 128.19, 128.13, 127.92, 119.41, 114.41, 55.38; MS (ESI): *m/z* = 326.4 [M^+].

4-(4,5-Diphenyl-1*H*-imidazol-2-yl)phenol *N8*. M.p. 232–235 °C (lit. 232–233 °C, [31]); FTIR (KBr, cm^{-1}): 3425 (O–H), 3063 (Ar–H), 1647 (C=N), 1604 (C=C), 1288 (C–O); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 9.92 (*b*, 1H, NH), 7.95 (*d*, *J* = 6.7 Hz, 2H, ArH), 7.68–7.52 (*m*, 6H, ArH), 7.39 (*t*, *J* = 6.0 Hz, 2H, ArH), 7.33 (*d*, *J* = 5.8 Hz, 2H, ArH), 6.92 (*d*, *J* = 6.8 Hz, 2H, ArH); ^{13}C-NMR (125 MHz, DMSO-d_6) δ(ppm): 158.61, 145.62, 131.76, 131.04, 129.51, 129.43, 129.20, 128.66, 128.44, 128.28, 127.89, 127.52, 127.47, 119.53, 115.58; MS (ESI): *m/z* = 312.4 [M^+].

2-(3,4-Dimethoxyphenyl)-4,5-diphenyl-1*H*-imidazole *N9*. M.p. 220–221 °C (lit. 220–224 °C, [32]); FTIR (KBr, cm^{-1}): 3390 (N–H), 3063 (Ar–H), 1647 (C=N), 1600 (C=C), 1265 (C–O), 1153 (O–CH_3); ^{1}H-

NMR (500 MHz, DMSO-d_6) δ (ppm): 12.70 (*s*, 1H, NH) 7.74 (*d, J* = 6.9 Hz, 1H, ArH), 7.54 (*d, J* = 5.7 Hz, 1H, ArH), 7.46–7.39 (*m*, 10H, ArH), 7.17 (*d, J* = 6.5 Hz, 1H, ArH), 3.87, 3.85 (*s*, 3H, 2OCH$_3$); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 150.57, 148.92, 144.69, 130.32, 129.98, 128.63, 128.30, 128.21, 119.42, 118.86, 111.97, 109.63, 55.76, 55.68; MS (ESI): *m/z* = 356.4 [M$^+$].

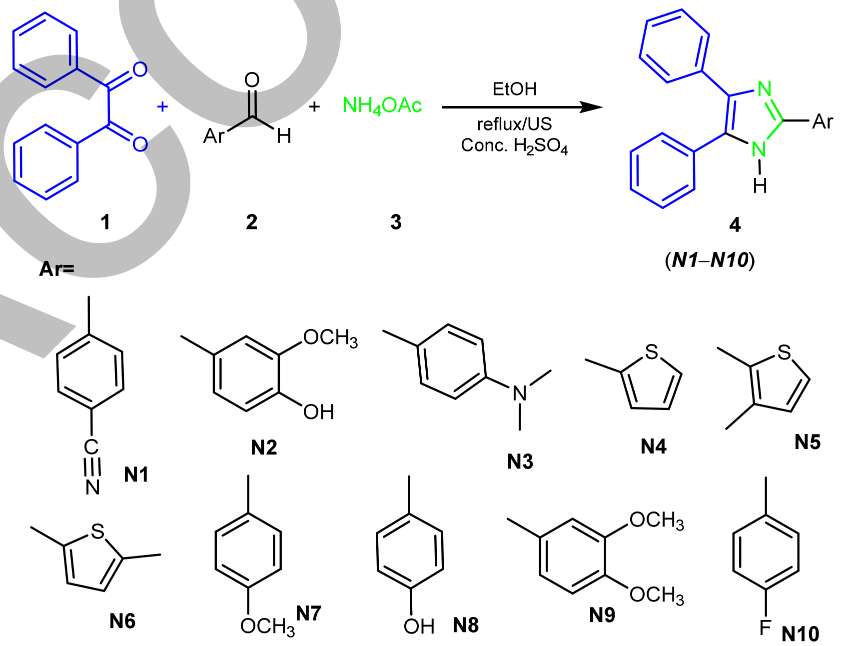
2-(4-Fluorophenyl)-4,5-diphenyl-1*H*-imidazole *N10*. M.p. 258–260 °C (lit. 256–260 °C, [33]); FTIR (KBr, cm^{-1}): 3317 (N–H), 3063 (Ar–C–H), 1674 (C=N), 1597 (C=C), 1215 (C–F). ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 13.03 (*s*, 1H, NH), 8.15–7.25 (*m*, 14H, ArH); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 167.24, 163.82, 159.32, 145.10, 136.02, 135.28, 129.45, 129.30, 129.17, 128.96, 128.83, 128.78, 128.52, 128.36, 128.26, 128.13, 127.58, 126.31, 125.97, 115.49, 115.31; MS (ESI): *m/z* = 314.2 [M$^+$].

■ RESULTS AND DISCUSSION

Synthesis and Evaluation of 2,4,5-Trisubstituted Imidazole Derivatives (*N1–N10*)

Ten 2,4,5-trisubstituted imidazole derivatives (*N1–N10*) were synthesized through a one-pot, three-component condensation involving benzil, aromatic aldehydes, and ammonium acetate in ethanol, and the characterization of FTIR, ^{1}H-, ^{13}C-NMR, and MS was provided in Fig. S1–S10. Ethanol was chosen as the reaction medium due to its ability to enhance product yield and efficiently facilitate proton transfer during condensation [34]. The general reaction mechanism is illustrated in (Scheme 1). Reaction optimization included a comparison between conventional reflux heating and ultrasound-assisted synthesis, employing concentrated sulfuric acid as a Brønsted acid catalyst for both approaches. The ultrasound-assisted method showed remarkable improvements in reaction efficiency [35]. Reaction times significantly dropped to 30–50 min, while product yields remained stable or increased slightly, ranging from 80 to 87%. In contrast, conventional heating took 9–12 h and typically resulted in lower yields of 68 to 76% for compounds ***N2***, ***N6***, and ***N10***. The exceptional performance of sonochemistry is probably due to cavitation effects and improved mass and energy transfer in the reaction medium.

Table 1 illustrates the operational and environmental benefits of the ultrasound-assisted technique. Compared to conventional heating, the ultrasound-assisted multicomponent protocol reduced reaction times from hours to minutes and utilized smaller amounts of a benign solvent (ethanol), resulting



Scheme 1. Synthesis of 2,4,5-trisubstituted imidazole derivatives (***N1–N10***)

Table 1. Comparison of the traditional and ultrasound irradiation methods for synthesizing 2,4,5-trisubstituted imidazole derivatives (***N1*–*N10***)

Entry	Product	Ultrasound condition		Conventional heating (reflux)	
		Time (min)	Yield (%)	Time (h)	Yield (%)
N1	4-(4,5-Diphenyl-1*H*-imidazol-2-yl)benzonitrile	50	82	11	76
N2	4-(4,5-Diphenyl-1*H*-imidazol-2-yl)-2-methoxyphenol	40	80	10	69
N3	4-(4,5-Diphenyl-1*H*-imidazol-2-yl)-*N*,*N*-dimethylaniline	30	85	9	71
N4	4,5-Diphenyl-2-(thiophen-2-yl)-1*H*-imidazole	30	82	11	74
N5	2-(3-Methylthiophen-2-yl)-4,5-diphenyl-1*H*-imidazole	30	80	11	72
N6	2-(5-Methylthiophen-2-yl)-4,5-diphenyl-1*H*-imidazole	45	80	10	68
N7	2-(4-Methoxyphenyl)-4,5-diphenyl-1*H*-imidazole	30	87	10	75
N8	4-(4,5-Diphenyl-1*H*-imidazol-2-yl)phenol	50	81	10	74
N9	2-(3,4-Dimethoxyphenyl)-4,5-diphenyl-1*H*-imidazole	45	85	11	76
N10	2-(4-Fluorophenyl)-4,5-diphenyl-1*H*-imidazole	50	83	12	70

in lower energy consumption and reduced waste generation. These features underline the green and energy-efficient nature of the method, in accordance with the principles of green chemistry [36]. The reactions were carried out in a laboratory ultrasonic cleaner bath (40 kHz), which provides a safe, uniform, and easily controlled application of ultrasound energy to small-scale reaction mixtures, minimizing localized overheating and ensuring reproducible conditions. Although ultrasonic cleaners are less common than probe systems in organic synthesis, several studies have successfully applied them to multicomponent and heterocyclic reactions under mild conditions, achieving yields that are comparable to, or even higher than, those obtained with conventional methods [37].

The structures of ***N1*–*N10*** were confirmed using FTIR, ^{1}H-NMR, ^{13}C-NMR, and MS. In the FTIR spectra, the disappearance of C=O and NH_2 stretches and the appearance of characteristic C=N bands at 1604–1674 cm^{-1} indicated successful cyclization. In the ^{1}H-NMR spectra, the absence of amino protons and the appearance of a singlet at 9.68–13.0 ppm (NH of imidazole) confirmed ring closure. Aromatic and thiophene protons appeared at 6.8–8.1 ppm range. Methoxy and methyl proton signals were observed in the ^{1}H-NMR spectra of ***N2***, ***N5***, ***N6***, and ***N9***. For ***N2***, the methoxy signal appeared at 3.88 ppm. In ***N5*** and ***N6***, the $-CH_3$ signals were observed at 2.53 and 2.50 ppm, respectively. Compound ***N9*** exhibited two distinct $-OCH_3$ signals at 3.87 and 3.85 ppm. The ^{13}C-NMR spectra showed typical imidazole C=N signals around 140–145 ppm and well-resolved aromatic and heteroaryl carbon peaks. Mass spectrometry confirmed molecular weights, and the sharp melting points indicated high purity. Complete spectral details are provided in the experimental section.

Biological Evaluation

For the biological evaluation, four derivatives (***N2***, ***N4***, ***N5*****,** and ***N8***) were selected because they represent the main structural diversity of the synthesized series, including phenolic groups with and without methoxy substitution as well as thiophene and methylthiophene heterocycles. This representative set allowed us to examine the effects of heterocyclic substitutions and electron-donating groups on hemocompatibility and anti-inflammatory activity, providing an initial screening and a preliminary understanding of structure–activity relationships rather than a comprehensive biological evaluation at this stage.

Protein Inhibition Assay

To evaluate the anti-inflammatory potential of the derivatives, their ability to inhibit trypsin, a serine protease involved in inflammation, was assessed. As illustrated in Fig. 1, all compounds exhibited an apparent concentration-dependent inhibitory effect. Among them,

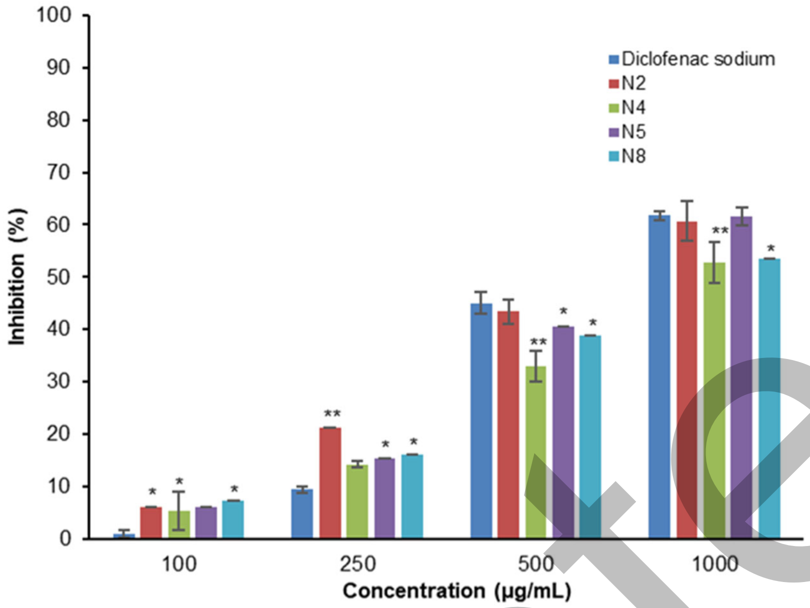


Fig 1. Effect of synthesized 2,4,5-trisubstituted imidazole derivatives on proteinase inhibitory activity. Diclofenac sodium was used as a reference standard (positive control). Significant differences compared to control were indicated as $^{*}p < 0.05$ and $^{**}p < 0.01$

N2 displayed the most insignificant activity (60.71 ± 3.68% at 1000 µg/mL), closely followed by ***N5*** (61.63 ± 1.74%). These values nearly matched the standard drug diclofenac inhibition level (61.69 ± 0.74%), suggesting strong protease-binding efficiency, whereas ***N8*** demonstrated intermediate inhibition (53.49 ± 0.45% at 1000 µg/mL), which could be attributed to steric hindrance from its bulky phenolic substituent [37]. Besides ***N4***, which has the lowest inhibition (50.33 ± 0.58%), may suffer from weaker electronic or spatial complementarity with the enzyme's active site. These results emphasize the importance of both hydrophobicity and electronic effects of substituents in modulating inhibitory strength. The 3-methylthiophene and methoxy groups in ***N5*** and ***N2*** seem to enhance π-stacking or hydrogen bonding with the enzyme, providing structural advantages for enzyme interaction [38].

Albumin Denaturation Assay

The ability of the compounds to prevent thermal denaturation of bovine serum albumin—a model for assessing anti-inflammatory behavior was also investigated. As shown in Fig. 2, all compounds demonstrated significant activity, with varying degrees of effectiveness. ***N8*** (76.55 ± 0.89%) and ***N5*** (76.8 ± 6.3%) exhibited the highest inhibition at 1000 µg/mL, approaching the standard diclofenac (83.33 ± 6.6%). ***N2*** also exhibited moderate effectiveness (58.33 ± 0.62%), while ***N4*** showed the lowest inhibition (52.84 ± 3.92%). The data suggest that polar functional groups, such as the phenolic hydroxyl in ***N8*** and the methoxy group in ***N2***, can stabilize protein structures under denaturing conditions, thereby enhancing their anti-inflammatory properties [39].

Compounds bearing electron-donating groups, such as methoxy and hydroxyl moieties on the phenyl ring, demonstrate enhanced biological activity, likely due to their ability to promote hydrogen bonding or electrostatic interactions with biological targets. In contrast, the less polar thiophene-containing derivatives (***N4*** and ***N5***) show moderate activity. Notably, ***N5*** demonstrates improved performance, likely due to the methyl substitution that enhances hydrophobic interactions. Both ***N8*** and ***N5*** demonstrate a favorable balance between therapeutic effectiveness and biological safety, characterized by adequate protein protection and low hemolytic activity [40]. Awasthi et al. [12] reported

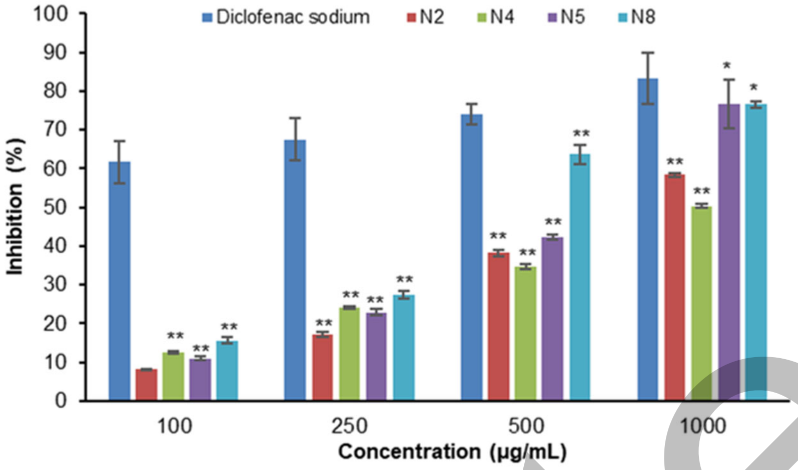


Fig 2. Effect of synthesized 2,4,5-trisubstituted imidazole on heat-induced albumin denaturation. Diclofenac sodium was used as the reference standard (positive control). Significant differences were indicated as $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to the control

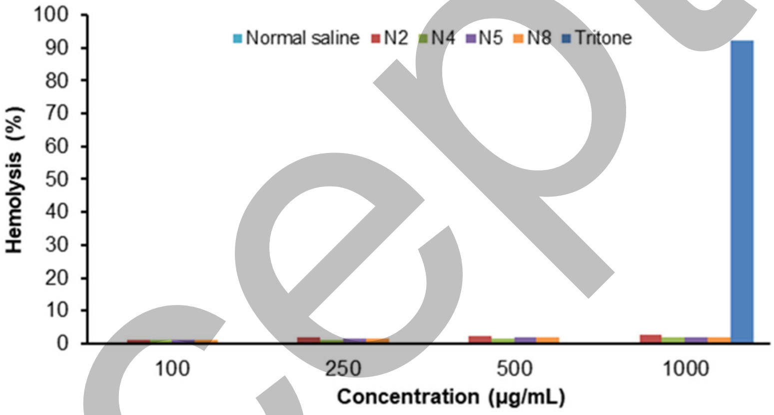


Fig 3. Hemolytic activities of ***N4***, ***N5***, ***N8***, and ***N12*** at the concentrations of 100, 250, 500, and 1000 μg/mL

that imidazole compounds exhibited a significant reduction in BSA denaturation, indicating an anti-inflammatory action. Likewise, our synthesized 2,4,5-trisubstituted imidazole derivatives exhibited similar inhibition, reinforcing their potential as anti-inflammatory agents.

Hemolysis Assay

The hemolysis test was assessed to determine the impact of the synthesized ***N2***, ***N4***, ***N5***, and ***N8*** on blood. As illustrated in Fig. 3, all compounds exhibited low hemolytic activity dependent on concentration. At the maximum concentration tested (1000 μg/mL), the hemolysis percentages ranged from 1.882% for ***N4*** to 2.58% for ***N2***, signifying minimal damage to red blood cell membranes. In contrast, the positive control (Triton X-100) caused hemolysis greater than 90%, while normal saline resulted in almost no hemolysis, thereby validating the assay. The minimal disruption suggests that these compounds do not have membrane-destabilizing properties, which supports their safe use in systemic applications. Notably, ***N4*** and ***N5***, which contain thiophene substituents, showed slightly lower hemolytic activity, likely due to weaker polar surface interactions. Overall, the findings support the hemocompatibility of the tested derivatives, a critical property for the safe systemic application of anti-inflammatory agents [41-42]. Recent studies have rarely integrated hemolysis testing with the anti-inflammatory assessment of heterocyclic compounds. COMA4-based coumarins demonstrated significant erythrocyte compatibility, potent anti-inflammatory activity, and clear structure–activity relationships [43], underscoring the validity of our evaluation method for imidazole derivatives.

■ CONCLUSION

This study aimed to synthesize and evaluate 2,4,5-trisubstituted imidazole derivatives using an ultrasound-assisted method. The synthesized compounds showed good anti-inflammatory activity and blood compatibility. These results indicate that the ultrasound-assisted method is efficient and environmentally benign, adhering to the principles of green chemistry. Overall, this work supports the potential of imidazole-based scaffolds in developing anti-inflammatory agents and recommends further investigations of their activity in biological systems.

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■ CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest, financial, personal, or otherwise, that could have influenced the outcomes of this research.

■ AUTHOR CONTRIBUTIONS

Duaa Abed Hilal carried out all experimental work, performed data analysis and prepared and revised the initial manuscript draft. Haitham Dalol Hanoon and Narjis Hadi Al-Saadi validated the research findings and supervised the overall project. All authors have approved the final version of the manuscript.

■ REFERENCES

[1] Abbasov, V.M., Marzouk, A.A., Mammadov, A.M., Kazimova, S.Z., and Talybov, A.H., 2012, Imidazole derivatives: Synthesis and biological activity, *Processes Petrochem. Oil Refin.*, 13 (4), 347–364.

[2] Siwach, A., and Verma, P.K., 2021, Synthesis and therapeutic potential of imidazole containing compounds, *BMC Chem.*, 15 (1), 12.

[3] Zarenezhad, E., Behrouz, S., Behrouz, M., and Rad, M.N.S., 2024, Synthesis, antimicrobial, antifungal and *in silico* assessment of some 2,4,5-trisubstituted imidazole analogues, *J. Mol. Struct.*, 1296, 136839.

[4] Vijesh, A.M., Isloor, A.M., Telkar, S., Arulmoli, T., and Fun, H.K., 2013, Molecular docking studies of some new imidazole derivatives for antimicrobial properties, *Arabian J. Chem.*, 6 (2), 197–204.

[5] Hussein, B.R.M., Mohammed, H.H., Ahmed, E.A., and Omran, O.A., 2025, One-pot multicomponent reaction: Synthesis, characterization and antibacterial evaluation of novel tri- and tetra-substituted imidazoles, *Synth. Commun.*, 55 (3), 270–280.

[6] Tolomeu, H.V., and Fraga, C.A.M., 2023, Imidazole: Synthesis, functionalization and physicochemical properties of a privileged structure in medicinal chemistry, *Molecules*, 28 (2), 838.

[7] Goel, T., Bansode, D., Abdu, R.A., and Dev, S., 2023, Molecular docking, microwave-assisted synthesis, characterization and pharmacological evaluation of 2,4,5-trisubstituted imidazoles, *Curr. Microwave Chem.*, 10 (1), 43–52.

[8] Baechle, J.J., Chen, N., Makhijani, P., Winer, S., Furman, D., and Winer, D.A., 2023, Chronic inflammation and the hallmarks of aging, *Mol. Metab*, 74, 101755.

[9] Leszek, J., Barreto, G.E., Gąsiorowski, K., Koutsouraki, E., Ávila-Rodrigues, M., and Aliev, G., 2016, Inflammatory mechanisms and oxidative stress as key factors responsible for progression of neurodegeneration: role of brain innate immune system, *CNS Neurol. Disord.: Drug Targets*, 15 (3), 329–336.

[10] Solleiro-Villavicencio, H., and Rivas-Arancibia, S., 2018, Effect of chronic oxidative stress on neuroinflammatory response mediated by CD4$^+$T cells in neurodegenerative diseases, *Front. Cell. Neurosci.*, 12, 114.

[11] Husain, A., Drabu, S., Kumar, N., Alam, M.M., and Bawa, S., 2013, Synthesis and biological evaluation of di- and tri-substituted imidazoles as safer anti-inflammatory-antifungal agents, *J. Pharm. BioAllied Sci.*, 5 (2), 154–161.

[12] Awasthi, A., Rahman, M.A., and Bhagavan Raju, M., 2023, Synthesis, *in silico* studies, and *in vitro* anti-inflammatory activity of novel imidazole derivatives targeting p38 MAP kinase, *ACS Omega*, 8 (20), 17788–17799.

[13] Singh, D., Kharb, R., and Sharma, S.K., 2024, Schiff's base imidazole derivatives synthesis and evaluation for their anti-inflammatory activity, *Orient. J. Chem.*, 40 (1), 152–164.

[14] Wang, L.M., Wang, Y.H., Tian, H., Yao, Y.F., Shao, J.H., and Liu, B., 2006, Ytterbium triflate as an efficient catalyst for one-pot synthesis of substituted imidazoles through three-component condensation of benzil, aldehydes and ammonium acetate, *J. Fluorine Chem.*, 127 (12), 1570–1573.

[15] Husain, A., AlAsmari, A.F., Azmi, S.N.H., Ali, N., Sarker, M.M.R., Alharbi, M., Ishtikhar, M., and Khan, S.A., 2022, Rational drug design, synthesis, and in vivo biological activity of new indolyl-imidazolone hybrids as potential and safer non-steroidal anti-inflammatory agents, *J. King Saud Univ., Sci.*, 34 (4), 102023.

[16] Gajengi, A.L., Chaurasia, S., Monflier, E., Ponchel, A., Ternel, J., and Bhanage, B.M., 2021, Ultrasound-assisted synthesis of NiO nanoparticles and their catalytic application for the synthesis of trisubstituted imidazoles under solvent-free conditions, *Catal. Commun.*, 161, 106366.

[17] Borhade, A.V., Tope, D.R., and Gite, S.G., 2017, Synthesis, characterization and catalytic application of silica supported tin oxide nanoparticles for synthesis of 2,4,5-tri- and 1,2,4,5-tetrasubstituted imidazoles under solvent-free conditions, *Arabian J. Chem.*, 10 (Suppl. 1), S559–S567.

[18] Sakhdari, M., Amoozadeh, A., and Kolvari, E., 2021, Magnetic nanoparticle-supported sulfonic acid as a green catalyst for the one-pot synthesis of 2,4,5-trisubstituted imidazoles and 1,2,4,5-tetrasubstituted imidazoles under solvent-free conditions, *Heterocycl. Commun.*, 27 (1), 71–78.

[19] Das, P.J., Das, J., Ghosh, M., and Sultana, S., 2013, Solvent-free one-pot synthesis of 1,2,4,5-tetrasubstituted imidazoles catalyzed by secondary amine-based ionic liquid and defective Keggin heteropoly acid, *Green Sustainable Chem.*, 3 (4A), 6–13.

[20] Zhang, F., Gao, Q., Chen, B., Bai, Y., Sun, W., Lv, D., and Ge, M., 2016, A practical and green approach towards synthesis of multisubstituted imidazoles using boric acid as efficient catalyst, *Phosphorus, Sulfur Silicon Relat. Elem.*, 191 (5), 786–789.

[21] Ahmed, N.S.H., and Hanoon, H.D., 2021, A green and simple method for the synthesis of 2,4,5-trisubstituted-1*H*-imidazole derivatives using acidic ionic liquid as an effective and recyclable catalyst under ultrasound, *Res. Chem. Intermed.*, 47 (10), 4083–4100.

[22] Tambe, A., Gadhave, A., Pathare, A., and Shirole, G., 2021, Novel pumice@SO_3H catalyzed efficient synthesis of 2,4,5-triarylimidazoles and acridine-1,8-diones under microwave-assisted solvent-free path, *Sustainable Chem. Pharm.*, 22, 100485.

[23] Alshook, N.A., Mihsen, H.H., and Hanoon, H.D., 2024, Synthesis and characterization of solid Brønsted acid derived from rice husks as an efficient catalyst for the preparation of some 2,4,5-trisubstituted imidazole derivatives, *Res. Chem. Intermed.*, 50 (6), 2581–2601.

[24] Alshook, N.A., Hanoon, H.D., and Mihsen, H.H., 2025, Development of a silica–organic framework ionic solid Brønsted acid catalyst for the synthesis of 2,4,5-trisubstituted imidazole derivatives, *J. Turk. Chem. Soc., Sect. A*, 12 (1), 1–14.

[25] Marques, M.V., Ruthner, M.M., Fontoura, L.A.M., and Russowsky, D., 2012, Metal chloride hydrates as Lewis acid catalysts in multicomponent synthesis of 2,4,5-triarylimidazoles or 2,4,5-triaryloxazoles, *J. Braz. Chem. Soc.*, 23 (1), 171–179.

[26] Banothu, J., Gali, R., Velpula, R., and Bavantula, R., 2017, Brønsted acidic ionic liquid catalyzed an efficient and eco-friendly protocol for the synthesis of 2,4,5-trisubstituted-1*H*-imidazoles under solvent-free conditions, *Arabian J. Chem.*, 10 (Suppl. 2), S2754–S2761.

[27] Okda, H.E., El Sayed, S., Ferreira, R.C.M., Costa, S.P.G., Raposo, M.M., Martínez-Máñez, R., and

Sancenón, F., 2018, 4-(4,5-Diphenyl-1*H*-imidazole-2-yl)-*N*,*N*-dimethylaniline–Cu(II) complex: A highly selective probe for glutathione sensing in water–acetonitrile mixtures, *Dyes Pigm.*, 159, 45–48.

[28] Gurav, S.S., Jadhav, S.R., Mali, S.N., Lotlikar, O.A., and Waghmode, K.T., 2023, An efficient one-pot multicomponent, Amberlite IR120(H) catalyzed microwave-assisted synthesis of 1,2,4,5-tetrasubstituted-1*H*-imidazoles: Plausible mechanism and antibacterial evaluation, *Synth. Commun.*, 53 (23), 2029–2040.

[29] Eseola, A.O., Görls, H., Orighomisan Woods, J.A., and Plass, W., 2020, Single monodentate N-donor ligands versus multi-ligand analogues in Pd(II)-catalysed C–C coupling at reduced temperatures, *Polyhedron*, 182, 114507.

[30] Pervaiz, S., Mutahir, S., Ullah, I., Ashraf, M., Liu, X., Tariq, S., Zhou, B.J., and Khan, M.A., 2020, Organocatalyzed solvent-free and efficient synthesis of 2,4,5-trisubstituted imidazoles as potential acetylcholinesterase inhibitors for Alzheimer's disease, *Chem. Biodiversity*, 17 (3), e1900493.

[31] Samai, S., Nandi, G.C., Singh, P., and Singh, M.S., 2009, L-Proline: An efficient catalyst for the one-pot synthesis of 2,4,5-trisubstituted and 1,2,4,5-tetrasubstituted imidazoles, *Tetrahedron*, 65 (49), 10155–10161.

[32] Shelke, K.F., Sapkal, S., Sonal, S., Madje, B.R., Shingate, B.B., and Shingare, M.S., 2009, An efficient synthesis of 2,4,5-triaryl-1*H*-imidazole derivatives catalyzed by boric acid in aqueous media under ultrasound irradiation, *Bull. Korean Chem. Soc.*, 30 (5), 1057–1060.

[33] Asressu, K.H., Chan, C.K., and Wang, C.C., 2021, TMSOTf-catalyzed synthesis of trisubstituted imidazoles using hexamethyldisilazane as a nitrogen source under neat and microwave irradiation conditions, *RSC Adv.*, 11 (45), 28061–28071.

[34] Hilal, D.A., and Hanoon, H.D., 2020, Brønsted acidic ionic liquid catalyzed an eco-friendly and efficient procedure for synthesis of 2,4,5-trisubstituted imidazole derivatives under ultrasound irradiation and optimal conditions, *Res. Chem. Intermed.*, 46 (2), 1521–1538.

[35] Ahmed, N.S., and Hanoon, H.D., 2022, Using ultrasound direct synthesis of some 2,4,5-triarylimidazole derivatives, *AIP Conf. Proc.*, 2547 (1), 040003.

[36] Abbas, S.K., Hanoon, H.D., and Al-Saadi, N.H., 2023, Ultrasound-assistance one-pot synthesis of 1,2,4,5-tetrasubstituted imidazole derivatives and their *in vitro* anti-urolithiasis activities, *Chem. Data Collect.*, 46, 101053.

[37] Martínez, R.F., Cravotto, G., and Cintas, P., 2021, Organic sonochemistry: A chemist's timely perspective on mechanisms and reactivity, *J. Org. Chem.*, 86 (20), 13833–13856.

[38] Govindappa, M., Hemashekhar, B., Arthikala, M.K., Ravishankar Rai, V., and Ramachandra, Y.L., 2018, Characterization, antibacterial, antioxidant, antidiabetic, anti-inflammatory and antityrosinase activity of green synthesized silver nanoparticles using *Calophyllum tomentosum* leaves extract, *Results Phys.*, 9, 400–408.

[39] Ruiz-Ruiz, J.C., Matus-Basto, A.J., Acereto-Escoffié, P., and Segura-Campos, M.R., 2017, Antioxidant and anti-inflammatory activities of phenolic compounds isolated from *Melipona beecheii* honey, *Food Agric. Immunol.*, 28 (6), 1424–1437.

[40] Al-Jubouri, A.K., Al-Saadi, N.H., and Kadhim, M.A., 2022, Anti-inflammatory and antibacterial activity of copper nanoparticles synthesized from *Myrtus communis* leaves extract, *Iraqi J. Agric. Sci.*, 53 (3), 698–711.

[41] Azadbakht, M., Davoodi, A., Hosseinimehr, S.J., Keighobadi, M., Fakhar, M., Valadan, R., Faridnia, R., Emami, S., Azadbakht, M., and Bakhtiyari, A., 2020, Tropolone alkaloids from *Colchicum kurdicum* (Bornm.) Stef. (Colchicaceae) as the potent novel antileishmanial compounds; Purification, structure elucidation, antileishmanial activities and molecular docking studies, *Exp. Parasitol.*, 213, 107902.

[42] Babijczuk, K., Warżajtis, B., Starzyk, J., Mrówczyńska, L., Jasiewicz, B., and Rychlewska, U., 2023, Synthesis, structure and biological activity of indole–imidazole complexes with $ZnCl_2$: Can coordination enhance the functionality of bioactive ligands?, *Molecules*, 28 (10), 4132.

[43] Mustafa, Y.F., 2024, Combretastatin A4-based coumarins: Synthesis, anticancer, oxidative stress-relieving, anti-inflammatory, biosafety, and *in silico* analysis, *Chem. Pap.*, 78 (6), 3705–3720.