



Green Synthesis of Tetra Substituted Imidazole Using Microwave Irradiation and Evaluation of Anti-Inflammatory Activity

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ABSTRACT

Microwave Assisted Synthesis (MWAS) is a green and efficient technique that significantly reduces reaction time, solvent requirement and chemical waste. The present study focused on the green synthesis of 1,2,4,5-tetrasubstituted imidazole derivatives using microwave irradiation. From the literature survey, substituted imidazole derivatives were synthesized by a one-pot multicomponent condensation reaction using Benzyl, substituted aromatic aldehydes, Ammonium Acetate, para-Aminobenzoic acid and L-Proline. Five compounds, designated as TSI-1 to TSI-5, were synthesized under microwave irradiation at 80 °C and 200 W for 10 minutes. The purity of the synthesized compounds was determined by Thin Layer Chromatography (TLC), and the compounds were characterized by FT-IR and ¹H NMR spectral studies. The synthesized compounds showed good yields ranging from 78% to 89% with melting points of approximately 235–260 °C. All the synthesized compounds were evaluated for their in vitro anti-inflammatory activity by the albumin denaturation inhibition method using Diclofenac Sodium as the standard drug. Among the synthesized compounds, TSI-4 showed the highest inhibition of about 74% at 500 µg concentration, indicating promising anti-inflammatory activity.

KEYWORDS: Microwave Assisted Synthesis, Tetrasubstituted Imidazole Derivatives, Debus-Radziszewski imidazole synthesis, Green Synthesis, Anti-inflammatory activity, Albumin denaturation assay.

1. INTRODUCTION

1.1. Microwave Assisted Synthesis (MWAS):

Microwave Assisted Synthesis (MWAS) is an efficient technique used in synthetic organic chemistry to improve the speed and convenience of chemical reactions. The method uses microwave irradiation to provide rapid heating of the reaction mixture, which can reduce reaction time and solvent requirement. Microwave-assisted synthesis is also considered a useful green chemistry approach because it can help reduce energy consumption and chemical waste when compared with suitable conventional procedures.

1.1.1. General Considerations for Microwave Assisted Synthesis

1. Power: 100–1000 watts
2. Frequency: 2.45 GHz
3. Temperature: 50–300 °C
4. Reaction time: Few seconds to several minutes

1.1.2. Principle of Microwave Assisted Synthesis

Microwave irradiation produces heat through the interaction of the electromagnetic field with polar molecules and ionic species present in the reaction mixture. The molecules respond to the alternating microwave field, resulting in molecular movement and energy conversion into heat. This provides rapid heating of the reaction medium and may lead to faster completion of chemical reactions.

1.1.3. Mechanism of Microwave Assisted Synthesis:

The major mechanisms involved in microwave heating are:

(a) Dipolar polarization:

Polar molecules contain regions of partial positive and negative charges. During microwave irradiation, these dipoles continuously attempt to align with the alternating electromagnetic field, producing molecular movement and heat.

(b) Ionic conduction:

Ions present in the reaction medium move under the influence of the alternating microwave field. This movement produces energy in the form of heat and contributes to rapid heating.

(c) Interfacial polarization:

Interfacial polarization occurs at the boundary between different phases or materials. It involves the combined contribution of dipolar polarization and ionic conduction and can enhance microwave absorption and heating.

1.2. Imidazole

Imidazole is an important five-membered aromatic nitrogen-containing heterocyclic compound having two nitrogen atoms in its ring system. The imidazole nucleus possesses important chemical characteristics such as hydrogen-bonding ability, acid-base interactions and coordination properties. Because of these properties, imidazole derivatives have attracted considerable interest in medicinal chemistry. Various substituted imidazole's have been investigated for biological activities such as antimicrobial, antifungal and anti-inflammatory effects.

1.3. 1,2,4,5-Tetrasubstituted Imidazole

1,2,4,5-Tetrasubstituted imidazole's are an important class of substituted imidazole derivatives containing different substituents around the imidazole ring. The Debus-Radziszewski reaction is a classical multicomponent approach used for the preparation of substituted imidazole's. In the present study, benzyl, substituted aromatic aldehydes, ammonium acetate, para-aminobenzoic acid and L-proline were employed for the synthesis of tetrasubstituted imidazole derivatives using microwave irradiation.

This tetra substituted imidazole are designated as TSI-1 to TSI-5 and were synthesized using different aromatic aldehyde derivatives. The synthesized compounds were obtained as crystalline solid products and showed good, isolated yields. The compounds were further characterized by physical parameters, FT-IR and ^1H NMR spectral studies.

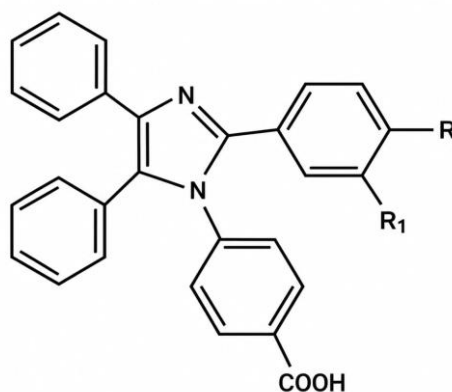


Figure 1. Structure of 1,2,4,5-Tetrasubstituted Imidazole.

2. EXPERIMENTAL SECTION

2.1 Materials:

Benzil, substituted aromatic aldehydes, para-aminobenzoic acid (PABA), ammonium acetate and L-proline were used for the synthesis of substituted imidazole derivatives. Ethanol was used as the reaction solvent and for recrystallization. Chloroform and ethyl acetate were used as the mobile phase for thin-layer chromatography (TLC).

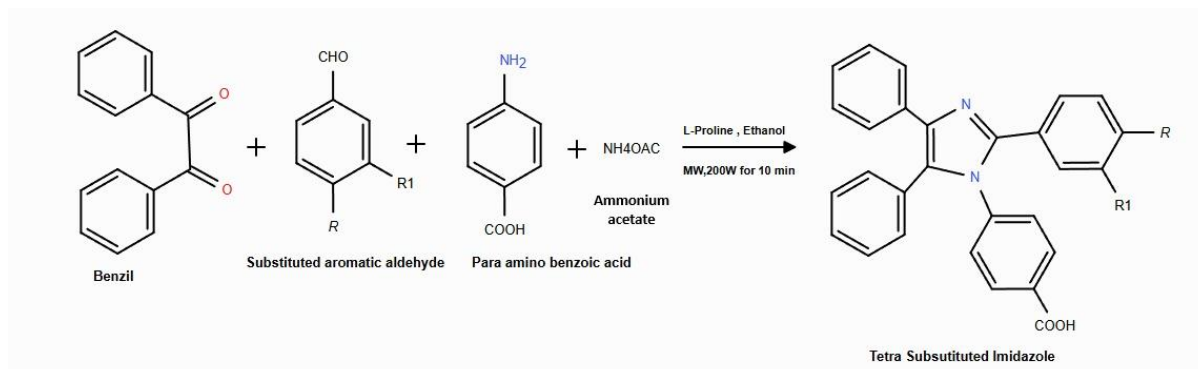
For the anti-inflammatory study, bovine serum albumin (BSA) and diclofenac sodium were used. Hydrochloric acid was used for pH adjustment.

2.2. Synthesis of 1,2,4,5-Tetrasubstituted Imidazole Derivatives⁽¹⁾

An equimolar mixture of Benzil (1 mmol) substituted aromatic aldehyde (1 mmol), Ammonium acetate (3 mmol), para-Aminobenzoic acid (1 mmol) and L-Proline (0.1 mmol) was placed in a 100 mL beaker and thoroughly mixed. The reaction mixture was subjected to microwave irradiation at 80 °C and 200 W for 10 minutes. The progress of the reaction was monitored by Thin Layer Chromatography (TLC) using chloroform: ethyl acetate (3:2) as the mobile phase.

After completion of the reaction, the reaction mixture was allowed to cool to room temperature and diluted with water. The resulting precipitate was collected by filtration and subsequently recrystallized from ethanol to obtain the purified 1,2,4,5-tetrasubstituted imidazole derivative. The obtained products were dried and used for further characterization and evaluation of anti-inflammatory activity.

Five, substituted imidazole derivatives were synthesized using different aromatic aldehydes, namely 4-Chlorobenzaldehyde, 4-Nitrobenzaldehyde, 4-Fluorobenzaldehyde, 4-Dimethylaminobenzaldehyde and 3-Nitrobenzaldehyde.



Scheme 2.1. Microwave-assisted synthesis of 1,2,4,5-tetrasubstituted imidazole derivatives

DESCRIPTION:

S.NO	BENZALDEHYDE DERIVATIVES	R1	R
1	TSI-1 4-[2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl] benzoic acid	H	Cl
2	TSI-2 4-[2-(4-fluorophenyl)-4,5-diphenyl-1H-imidazol-1-yl] benzoic acid	H	F

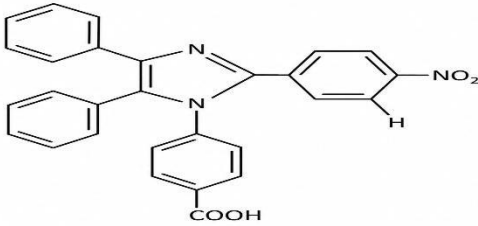
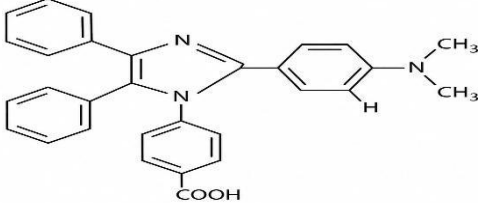
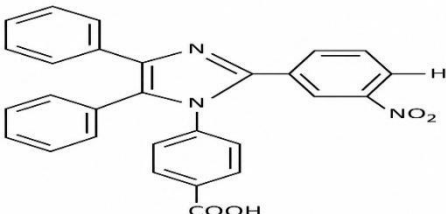
3	TSI-3  4-[2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazol-1-yl] benzoic acid	H	NO ₂
4	TSI-4  4-[2-[4-(dimethylamino)phenyl]-4,5-diphenyl-1H-imidazol-1-yl] benzoic acid	H	N(CH ₃) ₂
5	TSI-5  4-[2-(3-nitrophenyl)-4,5-diphenyl-1H-imidazol-1-yl] benzoic acid	NO ₂	H

Table:1

2.3 Anti-inflammatory Activity

Albumin Denaturation Inhibition Assay⁽¹⁾

The anti-inflammatory activity of the synthesized imidazole derivatives was evaluated by the albumin denaturation assay, using diclofenac sodium as the standard drug. The test compounds and standard drug were tested at concentrations of 250, 500 and 1000 µg/mL. For the assay, 0.45 mL of 1% aqueous bovine serum albumin (BSA) was mixed with 0.05 mL of the test solution or diclofenac sodium, making a total reaction volume of 0.5 mL. The pH of the reaction mixture was adjusted to 6.3 using dilute hydrochloric acid (HCl).

The reaction mixtures were incubated at 37°C for 20 minutes and subsequently heated at 55°C for 20 minutes. After heating, the samples were allowed to cool to room temperature. The resulting turbidity was measured at 660 nm using a UV-Visible spectrophotometer.

The percentage inhibition of albumin denaturation was calculated using the absorbance values obtained for the test samples and control. A higher percentage inhibition indicates better in-vitro anti-inflammatory activity.

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where,

A control = Absorbance of the control

A sample = Absorbance of the test sample

3. RESULT AND DISCUSSION

3.1. Physical Data:

NAME OF THE COMPOUND	APPEARANCE	MOLECULAR FORMULA	MOLECULAR WEIGHT	PERCENTAGE YEILD
4-[2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl]benzoic acid	Pale Orange	C ₂₈ H ₂₁ ClN ₂ O ₂	450.92 g/mol	85%
4-[2-(4-fluorophenyl) -4,5-diphenyl-1H-imidazol-1-yl] benzoic acid	Peach Orange	C ₂₈ H ₂₁ FN ₂ O ₂	434.47g/mol	89%
4-[2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazol-1-yl] benzoic acid	Reddish Orange	C ₂₈ H ₂₁ N ₃ O ₄	461.48g/mol	87%
4-[2[4(dimethyl amino) phenyl]-4,5-diphenyl-1H-imidazol-1-yl] benzoic acid	Dark brownish colour	C ₃₀ H ₂₆ N ₃ O ₂	459.55 g/mol	78%
4-[2-(3-nitrophenyl)-4,5-diphenyl-1H-imidazol-1-yl] benzoic acid	Light Peach	C ₂₈ H ₂₁ N ₃ O ₄	461.48g/mol	88%

Table:2

3.2. Spectral Data:

The synthesized compounds were characterized by various methods such as FT-IR and ¹H NMR.

TSI-1

Colour: pale orange; M.Pt: 245–250°C; FT-IR (ATR cm⁻¹): 1658.78 (C=O), 1593.20 (C=N), 3363.8 (O-H), 3062.96 (C-H), 713.66 (C-Cl); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.22–8.79 (Cl, 4H, Ar-H), 7.89–7.91 (2H, Ar-H [2',5']), 7.67–7.71 (3H, Ar-H [3',4',5']), 7.57–7.71 (2H, Ar-H [2',5']).

TSI-2

Colour: peach orange; M.Pt: 240–245°C; FT-IR (ATR cm⁻¹): 1658.78 (C=O), 1593.20 (C=N), 3360.00 (O-H), 1288.45 (F), 3062.96 (C-H); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.05–8.07 (o,2H, Ar-H), 8.03–8.05 (m, 2H, Ar-H), 7.59–7.83 [4H, Ar-H (2',6',2'',6'')], 7.42–7.57 (4H, Ar-H [3',5',3'',5'']), 7.09–7.18 (m, Ar-H).

TSI-3,

Colour: Reddish orange; M.Pt. 250–255°C; FT-IR (ATR cm⁻¹): 1658.78 (C=O), 1593.20 (C=N), 1516.05 (NO₂), 3062.96 (C-H); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.31–8.33 (o,2H, Ar-H), 8.01–8.15 (m, 2H, Ar-H), 7.91–7.99 (o,2H, Ar-H), 7.57–7.64 (4H, Ar-H [2',6',2'',6'']), 7.66–7.89 (4H, Ar-H [3',5',3'',5'']), 7.08–7.23 (m, 2H, Ar-H).

TSI-4

Colour: Dark brownish; M.Pt. 235–240°C; FT-IR (ATR cm⁻¹): 1658.78 (C=O), 1597.77 (C=N), 1161.15 (C-N), 2885.51 (C-H), 3062.96 (C-H); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.96–7.98 (o,2H, Ar-H), 7.13–7.25 (m, 2H, Ar-H), 6.69–6.71 (o,2H, Ar-H), 7.49–7.53 (4H, Ar-H [2',6',2'',6'']), 7.64–7.68 (4H, Ar-H [3',5',3'',5'']), 2.91–3.08 (6H, Ar-H).

TSI-5

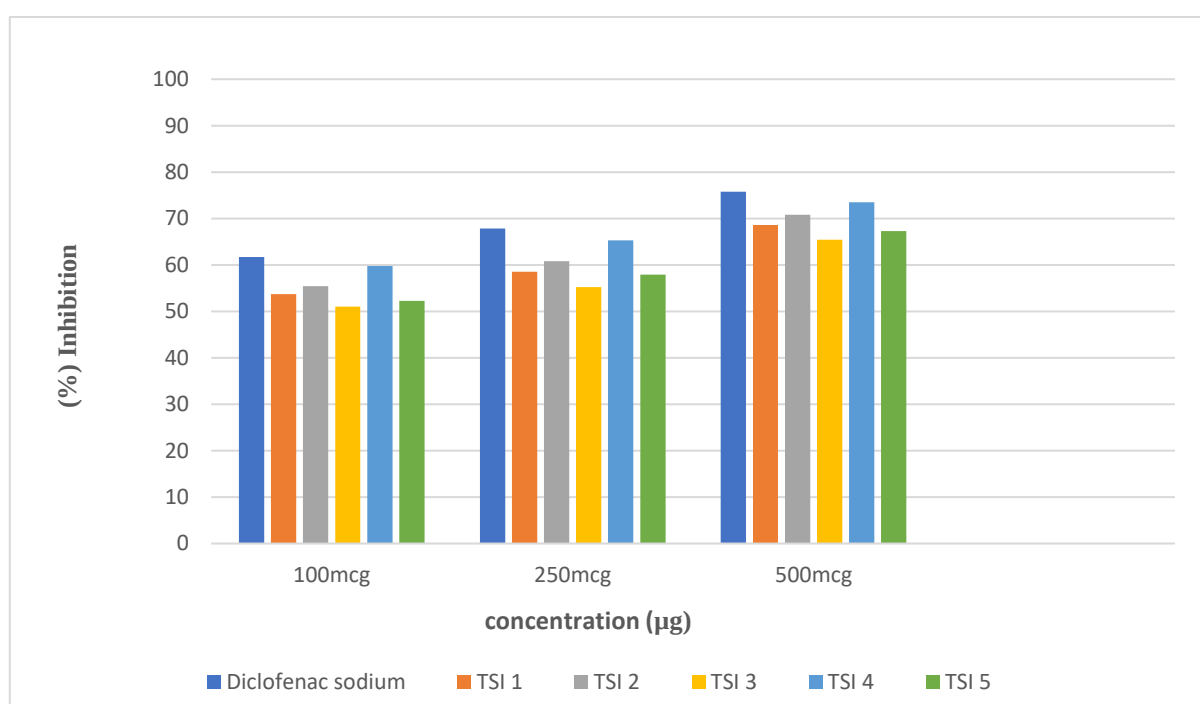
Colour: Light peach; M.Pt. 250–260°C; FT-IR (ATR cm⁻¹): 1654.92 (C=O), 1597.06 (C=N), 1519.91 (NO₂), 2630.91 (O-H), 3062.96 (C-H); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.41 (1H, Ar-H), 8.02–8.18 (o,2H, Ar-H), 7.81–7.91 (2H, Ar-H [5'',6'']), 7.57–7.65 (4H, Ar-H [2',6',2'',6'']), 7.67–7.76 (4H, Ar-H [3',5',3'',5'']), 7.09–7.11 (m, 2H, Ar-H).

3.3. ANTI-INFLAMMATORY ACTIVITY

Albumin Denaturation Inhibition assay:

COMPOUNDS	ACTIVITY ON 100µg	ACTIVITY ON 250µg	ACTIVITY ON 500µg
Diclofenac sodium	61.71	67.83	75.76
Tsi-1	53.73	58.51	68.59
Tsi-2	55.46	60.83	70.83
Tsi-3	50.99	55.25	65.42
Tsi-4	59.75	65.29	73.51
Tsi-5	52.28	57.94	67.32

Table:3



4. DISCUSSION:

The results demonstrate that microwave irradiation can be applied for the synthesis of substituted tetrasubstituted imidazole derivatives using a multicomponent approach. The synthesized compounds were obtained as crystalline solids with satisfactory isolated yields and characteristic melting points. FT-IR and ^1H NMR spectral data provided supportive evidence for the expected functional groups and structural features of the synthesized derivatives.

The albumin denaturation assay further showed concentration-dependent inhibition for all synthesized compounds. The observed activity indicates that the synthesized imidazole derivatives possess preliminary anti-inflammatory potential and provides a basis for further investigation of these compounds.

The synthesized TSI-1, TSI-2, TSI-3, TSI-4 and TSI-5 compounds were characterized by FT-IR spectroscopy to confirm the presence of their important functional groups. All the synthesized compounds showed characteristic absorption bands corresponding to C=O, C=N and aromatic C–H stretching vibrations. TSI-1 showed characteristic C=O, C=N, O–H and aromatic C–H absorption bands, while TSI-2 exhibited C=O, C=N, O–H, C–F and aromatic C–H absorption bands. TSI-3 and TSI-5 additionally showed characteristic NO₂ absorption bands, whereas TSI-4 exhibited a C–N stretching band. TSI-5 also showed an O–H absorption band. The observed FT-IR absorption frequencies were consistent with the proposed structures of the synthesized compounds. Thus, the FT-IR spectral results provided supportive evidence for the successful formation and structural confirmation of TSI-1 to TSI-5 derivatives.

The synthesized TSI-1, TSI-2, TSI-3, TSI-4 and TSI-5 compounds were characterized by ^1H NMR spectroscopy to confirm their proposed structures. The spectra showed signals mainly in the aromatic region (δ 6.6–8.4 ppm), corresponding to the protons of the aromatic rings. The observed signals and

their integration values were consistent with the expected number of aromatic protons in the synthesized derivatives. TSI-1 showed signals in the range of δ 7.57–8.22 ppm, while TSI-2 exhibited characteristic aromatic signals between δ 7.09–8.07 ppm. TSI-3 showed signals in the range of δ 7.08–8.33 ppm, indicating the presence of deshielded aromatic protons. TSI-4 exhibited signals mainly between δ 6.69–7.98 ppm, along with a signal around δ 2.91–3.08 ppm corresponding to aliphatic protons. TSI-5 displayed characteristic aromatic signals in the range of δ 7.09–8.41 ppm. The variations in chemical shifts were attributed to the different substituents present on the aromatic rings, which influence the electronic environment of the protons. Overall, the ^1H NMR spectral data were in agreement with the proposed structures and supported the successful synthesis of TSI-1 to TSI-5 derivatives.

5. CONCLUSION:

The 1,2,4,5-Tetrasubstituted imidazole derivatives were successfully synthesized by Microwave Assisted Synthesis using a green and efficient one-pot condensation method.

The synthesized compounds were purified by Thin Layer Chromatography (TLC) and recrystallization using ethanol.

The synthesized compounds were characterized by FT-IR and ^1H NMR spectral studies, which supported the formation of the desired imidazole derivatives.

The synthesized compounds showed satisfactory yields ranging from 78% to 89%. Among the synthesized compounds, TSI-2 showed the highest yield of 89%.

The synthesized compounds were evaluated for in vitro anti-inflammatory activity by the albumin denaturation inhibition method using Diclofenac Sodium as the standard drug.

Among the synthesized compounds, TSI-4 showed the highest anti-inflammatory activity, with approximately 74% inhibition at 500 $\mu\text{g/mL}$ concentration.

Overall, the present study demonstrates that Microwave Assisted Synthesis is a rapid and eco-friendly approach for the synthesis of tetrasubstituted imidazole derivatives with promising preliminary anti-inflammatory activity.

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7. REFERENCES:

1. Hilal DA, Al-Saadi NH, Hanoon HD. Synthesis of 1, 2, 4, 5-Tetrasubstituted Imidazoles via ultrasound-assisted method and in vitro assessment of their anti-inflammatory and Hemocompatibility activity. *Results in Chemistry*. 2026 Jan 8;103043.
2. Goudarzi H, Habibi D, Monem A. The protocatechuic acid-based deep eutectic solvent-mediated green synthesis of 1, 2, 4, 5-tetrasubstituted imidazoles. *RSC advances*. 2024 Jul 16;14(31):22459-69.
3. Duc DX, Lanh HT. Microwave-assisted,[Bmim] HSO₄-catalyzed synthesis of tetrasubstituted imidazoles via four-component reaction. *Vietnam Journal of Science and Technology*. 2022 Jul 8;60(3):383-90.
4. Ezhilmathi P, Thirumalai K, Swaminathan M, Krishnasamy K. One-Pot Synthesis of Tetra Substituted Imidazoles Catalyzed by Fly Ash Supported Bi₂O₃–ZnO. *Journal of Nanoscience and Nanotechnology*. 2019 Dec 1;19(12):8163-71.
5. Gabla JJ, Lathiya DR, Revawala AA, Maheria KC. Propyl–SO₃H functionalized SBA-15: Microwave-mediated green synthesis of biologically active multi-substituted imidazole scaffolds: JJ Gabla et al. *Research on Chemical Intermediates*. 2019 Apr 15;45(4):1863-81.
6. ÇEVİK UA, LEVENT S, ÖZKAY Y, KARACA H, CANTÜRK Z, TUNALI Y, UÇUCU Ü. Microwave assisted synthesis of some 1, 2, 4, 5-tetrasubstituted imidazole derivatives as antimicrobial agents. *Eur. Int. J. Sci. Technol*. 2016;5:59-76.
7. Shirole GD, Kadnor VA, Tambe AS, Shelke SN. Brønsted-acidic ionic liquid: green protocol for synthesis of novel tetrasubstituted imidazole derivatives under microwave irradiation via multicomponent strategy. *Research on Chemical Intermediates*. 2017 Feb;43(2):1089-98.
8. Chundawat TS, Sharma N, Kumari P, Bhagat S. Microwave-assisted nickel-catalyzed one-pot synthesis of 2, 4, 5-trisubstituted imidazoles. *Synlett*. 2016 Feb;27(03):404-8.
9. Safari J, Khalili SD, Banitaba SH. A novel and an efficient catalyst for one-pot synthesis of 2, 4, 5-trisubstituted imidazoles by using microwave irradiation under solvent-free conditions. *Journal of chemical sciences*. 2010 May;122(3):437-41.
10. Güngör T. Microwave assisted, sequential two-step, one-pot synthesis of novel imidazo [1, 2-a] pyrimidine containing tri/tetrasubstituted imidazole derivatives. *Turkish Journal of Chemistry*. 2021;45(1):219-30.
11. Balalaie S, Arabanian A. One-pot synthesis of tetrasubstituted imidazoles catalyzed by zeolite HY and silica gel under microwave irradiation. *Green Chemistry*. 2000 Jan 1;2(6):2746.

12. Safari J, Naseh S, Zarnegar Z, Akbari Z. Applications of microwave technology to rapid synthesis of substituted imidazoles on silica-supported SbCl_3 as an efficient heterogeneous catalyst. *Journal of Taibah University for Science*. 2014 Oct 1;8(4):323-30.
13. Karimi AR, Alimohammadi Z, Azizian J, Mohammadi AA, Mohammadizadeh MR. Solvent-free synthesis of tetrasubstituted imidazoles on silica gel/ NaHSO_4 support. *Catalysis Communications*. 2006 Sep 1;7(9):728-32.
14. Kantevari S, Vuppapapati SV, Biradar DO, Nagarapu L. Highly efficient, one-pot, solvent-free synthesis of tetrasubstituted imidazoles using HClO_4 - SiO_2 as novel heterogeneous catalyst. *Journal of Molecular Catalysis A: Chemical*. 2007 Apr 2;266(1-2):109-13.
15. Niknam K, Deris A, Naeimi F, Majleci F. Synthesis of 1, 2, 4, 5-tetrasubstituted imidazoles using silica-bonded propylpiperazine N-sulfamic acid as a recyclable solid acid catalyst. *Tetrahedron letters*. 2011 Sep 7;52(36):4642-5.
16. Rupčić R, Modrić M, Hutinec A, Čikoš A, Stanić B, Mesić M, Pešić D, Merćep M. Novel tetracyclic imidazole derivatives: Synthesis, dynamic NMR study, and anti-inflammatory evaluation. *Journal of heterocyclic chemistry*. 2010 May;47(3):640-56.
17. Atia AJ. Synthesis and antibacterial activities of new metronidazole and imidazole derivatives. *Molecules*. 2009 Jul 8;14(7):2431-46.
18. Sharma D, Narasimhan B, Kumar P, Judge V, Narang R, De Clercq E, Balzarini J. Synthesis, antimicrobial and antiviral evaluation of substituted imidazole derivatives. *European journal of medicinal chemistry*. 2009 Jun 1;44(6):2347-53.
19. Rossi R, Angelici G, Casotti G, Manzini C, Lessi M. Catalytic synthesis of 1, 2, 4, 5-tetrasubstituted 1H-imidazole derivatives: state of the art. *Advanced Synthesis & Catalysis*. 2019 Jun 18;361(12):2737-803.
20. Frantz DE, Morency L, Soheili A, Murry JA, Grabowski EJ, Tillyer RD. Synthesis of substituted imidazoles via organocatalysis. *Organic Letters*. 2004 Mar 4;6(5):843-6.