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GREEN SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF IMIDAZOLE DERIVATIVES

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ABSTRACT

The objective of the present investigation was to synthesize imidazole derivatives using microwave irradiation and evaluating them for antibacterial action. The synthesis was accomplished in two steps. While the first step was performed by conventional method, the second step that involved a modification of the triphenyl imidazole was performed using microwave irradiation for 30 to 120 seconds at 100 W power. The FTIR spectra of all the compounds exhibited stretching and bending vibrations for CH, C=N, C-N whereas N-O, C-O, C-Cl and C-Br stretching peaks were prominent in the corresponding compounds. The antibacterial activity of the synthesized compounds was determined using zone of inhibition method against gram positive (*S. aureus*, *B. subtilis*) and gram negative bacteria (*E. coli, Salmonella*). The presence of electron withdrawing groups in the compounds favored antibacterial activity against gram negative bacteria (5b, 5d & 5e) whereas electron donating group favored activity against gram positive bacteria (5b). Compound 5a did not exhibit significant antibacterial action as compared to the control (DMF).

KEYWORDS: Imidazole, microwave, antibacterial, FTIR, spectra.

INTRODUCTION

the research and developments of imidazole **(1)**-based compounds have been quite a rapidly developing and increasingly active area owing to their wide potential applications as medicinal drugs, agrochemicals, man-made materials, artificial acceptors, supramolecular ligands, biomimetic catalysts, and so on.[1] Imidazole derivatives are associated with many therapeutic fields. Some have been employed as Anthelmentics, Antibacterials & antiprotozoals. Miconazole, Clotrimazole, ketoconazole, econazole, tioconazole, bifonazole & terconazole are some currently used antifungal imidazole derivatives.[2-7] They all have activity against a broad range of microorganisms including both fungi & bacteria.

In light of the importance of the imidazole derivatives as antibacterial and anti fungal agents we planned to carry out the microwave assisted synthesis of some new imidazole derivatives and evaluate them for antimicrobial activity.

MATERIAL AND METHODS

Benzil was procured from Sulab; Ammonium acetate was purchased from Rankem, Aromatic aldehydes, glacial acetic acid and benzyl chloride were obtained from SD Fine Chemicals and Oxford Fine Chemicals. All other reagents and chemicals used for the synthesis were of synthetic grade and procured from Oxford Fine Chemicals. All the reagents and chemical were used as obtained without any further purification. All glassware was of borosilicate glass and cleaned using chromic acid cleaning mixture (conc. H_2SO_4 + sodium dichromate) before use.

The scheme for the synthesis of the imidazoles was adopted from the method reported by Hanoon et al[8] and modified for synthesis of derivatives (figure 1). The entire synthetic scheme was performed in two distinct steps:

1. Synthesis of substituted-2,4,5-triphenyl-1H-imidazole
2. Synthesis of substituted-1-benzyl-2,4,5-triphenyl-1H-imidazole

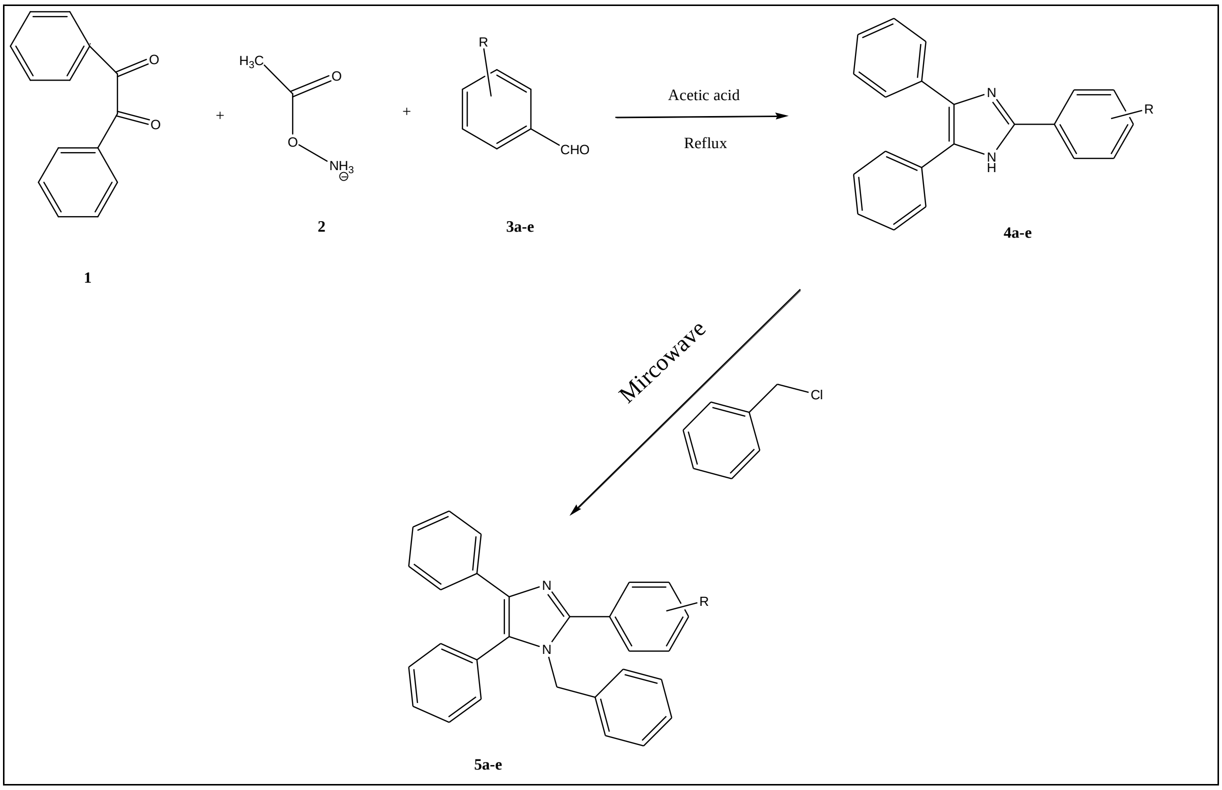

Figure 1: Scheme for synthesis of imidazole derivatives.

General method of synthesis of substituted 2,4,5-triphenyl-1H-imidazole

In to a clean dry round bottom flask, benzil (0.01 mol), ammonium acetate (0.05 mol), corresponding aromatic aldehyde (0.01 mol) and 25 ml of acetic acid were mixed. The mixture was refluxed for 1 h on a heating mantle. The reaction mixture was cooled and poured in to the 50 ml of water contained in a beaker. The precipitate that separated out was filtered and dried. The product was recrystallized from ethanol to obtain the 2,4,5-triphenyl-1H-imidazole. The reaction was monitored by TLC using (Methanol-dichloromethane, 1:1) as the solvent system.

General method for synthesis of substituted -1-benzyl-2,4,5-triphenyl-1H-imidazole

In a 100 ml beaker, substituted-2,4,5-triphenyl-1H-imidazole (0.01mol), benzyl chloride (0.01mol) and sodium hydroxide (0.01mol) were placed. 5 ml ethanol was added to this mixture and stirred. The mixture was heated in a microwave oven at 100W power and irradiated for 25 to 120 seconds. The reaction mixture was allowed to cool to obtain the product. The product was filtered, dried and recrystallized from ethanol to obtain the substituted final product. The reaction was checked for completion by TLC using (methanol:dichloromethane, 1:1) as the solvent system.

Physicochemical Characterization of the synthesized compounds

Melting points were determined using open capillary method and are uncorrected; solubility was determined qualitatively in various solvents. FTIR, 1HNMR and mass spectral studies were performed to confirm the structure of the compounds.

Antibacterial acivity

The compounds synthesized during the present investigation were screened for their antibacterial activity. The antibacterial tests were conducted on four common microorganisms viz. *Bacillus subtilis, Streptococcus aureus, Escherichia coli and Salmonella*, which are the representative types of gram positive and gram-negative organisms respectively. The antibacterial activity of the compounds was assessed by disc diffusion.

Preparation of Nutrient broth

- Nutrient broth powder – 37.2 g
- Distilled water - 1000 ml

Nutrient broth was prepared by dissolving all the ingredients and adjusting the pH adjusted to 7.2 and autoclaved at 15 lbs pressure for 20 min in an autoclave. One day before the testing, the microorganisms were subcultured into sterile nutrient broth and incubated at 37°C for 24 h. The culture growth thus obtained was used as inoculum for the antibacterial testing.

Preparation of nutrient agar media

The nutrient agar media was prepared by using the following ingredients.

- Nutrient agar – 28.6 g
- Distilled water - 1000 ml

The specified amount of nutrient agar powder was dissolved by heating on a water bath. and the volume of final solution is made up to 1000 ml with distilled water. The above prepared nutrient agar media was sterilized by autoclave at 121°C for 20 minutes at 15 lbs pressure.

Preparation of test solution

20 mg of the synthesized compounds were dissolved separately in 20 ml methanol. 1 ml of this solution was diluted to 10 ml with methanol. 0.5 ml (50 µg) and 1 ml (100 µg) of this solution was further diluted upto 2 ml by addition of methanol to obtain a solution of 25 and 50 µg/ml strength. These sample solutions were sterilized test tubes. These test compounds (25, 50 and 100 µg/ml) were soaked on small circular disc of 5 mm.

Preparation of standard solution

Ciprofloxacin was used as the standard drug at concentration of 50 and 100µg/ml prepared in distilled water.

***Procedure of antibacterial testing*[9]**

The sterilized media (nutrient agar) was cooled to 45°C with gentle shaking for uniform cooling and then inoculated with 18-24 h old bacterial subculture under aseptic conditions in a laminar air flow bench and mixed well by gentle shaking. This was poured in to sterile Petri dishes and allowed to set. After solidification all the Petri dishes were transferred to laminar flow unit and the test sample discs were carefully kept on the solidified media by using sterilized forceps. These Petri dishes were kept in the laminar air flow unit undisturbed for one-hour diffusion at room temperature and then for incubation at 37°C for 24 h in an incubator. The extent diameter of inhibition after 24 h was measured as the zone of inhibition in millimeters (mm).

RESULTS AND DISCUSSION

Chemistry

The scheme was optimized by varying the molar concentrations of the reactants and the reaction time in order to achieve maximum yield for the compounds. The optimized conditions comprised of 1:5:1 ratio of benzyl-ammonium acetate and aldehyde.

The R_f value obtained from TLC, melting point and percent yield of the synthesized compounds is depicted in Table 1. The solubility reveals that all the synthesized compounds were soluble in methanol and chloroform whereas insoluble in water and DMSO.

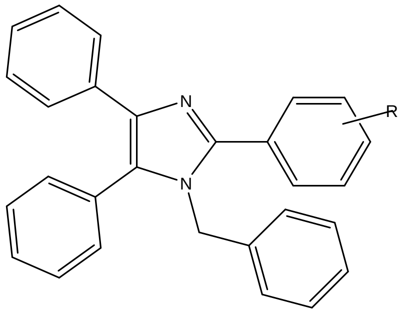

Table 1: physicochemical characters of imidazole derivatives.

Compound Code	R	Color	R_f Value	Melting Point	% Yield
5a	H	Pale Yellow	0.56	293-295°C	61
5b	NO_2	Yellow	0.67	268-271°C	57
5c	OH	Pale Yellow	0.51	276-278°C	64
5d	Cl	Brown	0.72	284-287°C	67
5e	Br	Yellow	0.75	290-293°C	59

The spectral studies (NMR, Mass and IR) were conducted to confirm the structure of the synthesized compounds. The spectra were obtained for the samples and the interpretation of each spectrum was carried out to ascertain the formation of desired bonds and incorporation of the functional groups. The FTIR spectra of all the compounds exhibited stretching and bending vibrations for CH, C=N, C-N whereas N-O, C-O, C-Cl and C-Br stretching peaks were prominent in the corresponding compounds. The peak for OH stretching was evident in compound 5c. The proton NMR spectra yielded shifts for aromatic protons as well as the CH_2 proton of the benzyl group. The proton for OH was almost merged with the CH_2 proton. The mass spectra revealed molecular ion peaks and isotopic peaks corresponding to the molecular mass of the compounds.

1-benzyl-2,4,5-triphenyl-1H-imidazole

IR (cm^{-1}): 2751 (C-H stretching), 1706 (C=N stretching), 1603 (C=C stretching), 1475 (C-H bend), 1316 (C-N stretching); ^{1}HNMR (δ ppm): 7.1-7.8 (CH, aromatic), 4.99 (CH_2); Mass (m/z): 386.4 (M^+ peak)

1-benzyl-2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazole

IR (cm^{-1}): 2756 (C-H stretching), 1699 (C=N stretching), 1603 (C=C stretching), 1521 (N-O stretching), 1480 (C-H bend), 1318 (C-N stretching); ^{1}HNMR (δ ppm): 6.8-7.9 (CH, aromatic), 8.01 (CH, adj to NO_2), 4.35 (CH_2); Mass (m/z): 432.2 (M+1 peak)

4-(1-benzyl-4,5-diphenyl-1H-imidazol-2-yl)phenol

IR (cm^{-1}): 3413 (O-H stretching), 2755 (C-H stretching), 1713 (C=N stretching), 1586 (C=C stretching), 1481 (C-H bend), 1323 (C-N stretching), 1079 (C-O stretching); ^{1}HNMR (δ ppm): 7.1-7.9 (CH, aromatic), 6.9 (CH, adjacent to OH), 4.99 (OH, CH_2); Mass (m/z): 402.1 (M^+ peak)

1-benzyl-2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole

IR (cm^{-1}): 2759 (C-H stretching), 1682 (C=N stretching), 1575 (C=C stretching), 1462 (C-H bend), 1321 (C-N stretching), 802 (C-Cl stretching); ^{1}HNMR (δ ppm): 7.1-7.8 (CH, aromatic), 4.85 (CH_2); Mass (m/z): 421.1 (M^+ peak)

1-benzyl-2-(4-bromophenyl)-4,5-diphenyl-1H-imidazole

IR (cm^{-1}): 3026 (C-H stretching), 1680 (C=N stretching), 1589 (C=C stretching), 1484 (C-H bend), 1313 (C-N stretching), 682 (C-Br stretching); ^{1}HNMR (δ ppm): 7.1-7.8 (CH, aromatic), 4.89 (CH_2); Mass (m/z): 466.1 (M+1 peak).

Antibacterial Action

The antibacterial activity of the synthesized imidazoles was determined measuring the zone of inhibition in the agar plate. Three concentrations of the synthesized compounds were tested for antibacterial action against ciprofloxacin as the standard drug for antibacterial action. The zone of inhibition of the test compounds is presented in table 2.

Table 2: Antibacterial activity of synthesized compounds.

Compound Code	**Zone of Inhibition (mm)***											
	B. subtilis			***S. auerus***			***E.coli***			***Salmonella***		
	25µg	**50µg**	**100µg**	**25µg**	**50µg**	**100µg**	**25µg**	**50µg**	**100µg**	**25µg**	**50µg**	**100µg**
5a	4	6	7	4	7	10	6	8	12	7	10	13
5b	6	9	13	5	9	14	10	15	**23**	9	15	**24**
5c	9	15	**24**	8	16	**25**	6	9	13	6	8	13
5d	6	9	13	5	9	14	10	16	**25**	9	16	**24**
5e	6	8	12	6	10	13	11	14	**23**	10	15	**23**
Ciprofloxacin	15	**21**	**33**	13	**22**	**27**	15	**23**	**35**	16	**22**	**36**

* Below 12 mm – poor activity; 13-18 mm – moderate activity & above 18 mm – good activity

The results revealed that the antibacterial action of the synthesized compounds was dose dependent. The compounds were mild to moderately antibacterial. The presence of electron withdrawing groups in the compounds favored antibacterial activity against gram negative bacteria (5b, 5d & 5e) whereas electron donating group favored activity against gram positive bacteria (5b). Compound 5a did not exhibit significant antibacterial action as compared to the control (DMF).

The lack of significant activity in 5a signifies the importance of the substitution of ring attached to 2-position of imidazole nucleus for antibacterial action. The positional effect of the substitution on this ring was though not studied.

CONCLUSION

Microwave irradiation provides a quick approach to synthesize organic compounds. The imidazole derivatives synthesized using microwave irradiation was obtained in good yields in a very low reaction time. The synthesized compounds exhibited mild to moderate antibacterial action against gram negative and gram positive bacteria. The study of a congeneric series of imidazole derivatives, determining it IC_{50} values and performing QSAR analysis would be highly helpful in designing newer antimicrobial compounds that would overcome the problems of resistance to antibiotics by the microbes.

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