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Short Communication

Microwave assisted synthesis of imidazolyl fluorescent dyes as antimicrobial agents

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ABSTRACT

In this article, we report, the synthesis and characterization of series of novel fluorescent imidazolyl dyes **(5a-d)** *via* highly efficient and cost-effective microwave assisted protocol as a potential candidate to overcome the problem of microbial resistance. By utilizing the green microwave protocol the reactions are completed in a short span of time without using the harsh conditions. The incorporation of imidazole nucleus is an important strategy in drug discovery. While designing desired fluorescent imidazole dyes **5a-d**, with a suitable auxiliary donor such as aromatic rings and $-OCH_3$ group on one end of the imidazolyl moiety and electron acceptors such as $-NO_2$ and $-COOH$ on other end of the compounds was achieved to get a promising fluorescent dyes for antimicrobial. The optoelectronic properties and antimicrobial studies of the synthesized materials indicated their exploration as a promising candidate as antimicrobial agents.

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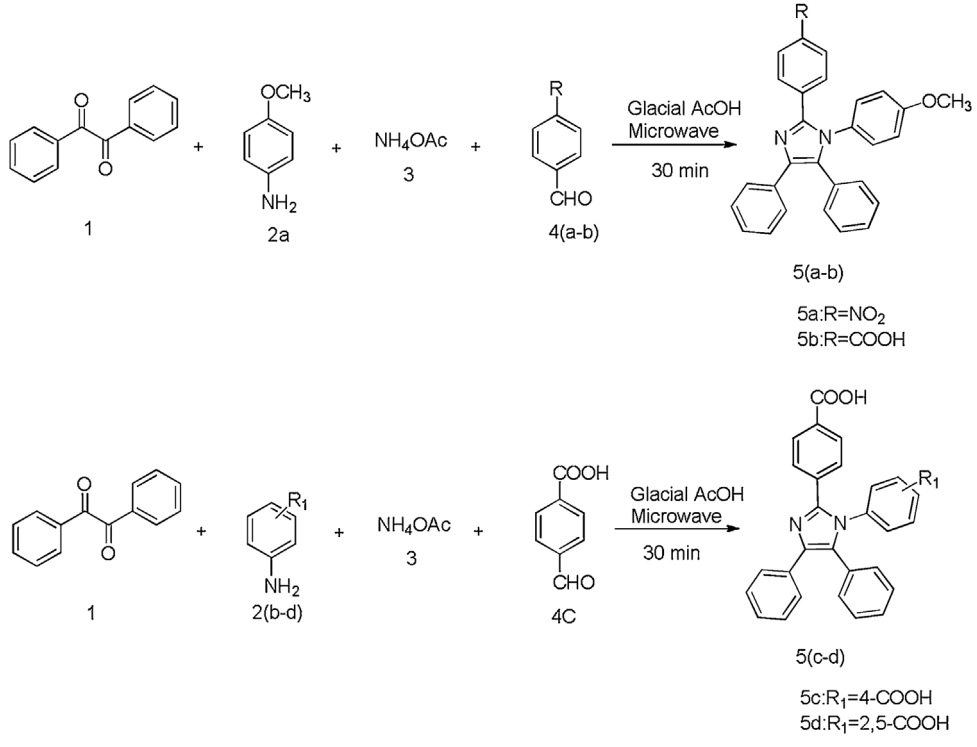
1. Introduction

Heteroatom containing organic materials as microbial agents have attracted great attention. The widespread development of antibiotic drug resistance over the past 10 years has resulted in a worldwide health crisis of global dimensions. Meanwhile, resistance rates around the world are rising, and infections caused by multidrug-resistant Gram-negative bacteria are becoming particularly difficult to treat. The most recent World Economic Forum Global Risks reports [1] have listed antibiotic resistance as one of the greatest threats to human health. In the literature various heterocyclic compounds are studied for antimicrobial activity viz., benzothiazepine [2], triazole [3], benzoxazole [4], indazole [5], quinazoline [6], pyrazole [7], imidazole and benzimidazole [8] etc.

Organic molecules with numerous structures reported to have wide range of application in lighting and display [9,10]. Among all the various heterocylic compounds, imidazole has become very significant owing to their numerous advantages in biological applications due to nitro herteroatom present in the skeleton [11,12]. Imidazoles such as 1-(4,5-diphenyl-1-p-tolyl-1H-imidazol-2-yl)naphthalen-2-ol, have been developed as a potential electronic materials [13]. Imidazole fluorescent compounds coupled with three different acceptor exhibited high stability [14,15]. An efficient deep blue fluorescent material based on pure aromatic structures has been reported wherein aromaticity increases the intensity increases [16–18]. Despite the influence of different acceptor groups on their photo-physical properties, due to their very sensitiveness in solvents environment, drastically changes their absorption and emission behaviors. In order to commercialize the drug with low cost and large-scale production mainly depends on the facile synthesis involving cost effective protocols [11].

Molecules containing carbazole, 4,5-diphenylimidazole, phenanthroimidazole triphenyl amine and tetraphenylethene have been synthesized and explored for non-doped and doped exhibited maximum external quantum efficiency [11,19,20]. Heterocylces with 2-(1, 4, 5-triphenyl-1H-imidazol-2-yl)phenol, exhibiting high thermal stability is explored by vacuum evaporation [21].

Thus, in the present study we aim to synthesize imidazole based florescent heterocyclic compounds to overcome the problem of microbial resistance. The incorporation of imidazole nucleus is an important strategy in drug discovery. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents based on imidazole nucleus. In continuation of our work [22–24] herein we developed a small organic Imidazolyl non-doped deep blue fluorescent dyes 5a-e *via* cost effective microwave protocol (Scheme 1) for application in microbial inhibition. Thus, while designing desired fluorescent imidazole dyes 5a-d, a suitable auxiliary donor such as aromatic rings and —OCH_3 group on one end of the imidazolyl moiety and electron acceptors such as —NO_2 and C—OOH on other end of the compounds was achieved to get to promising fluorescent materials as a probes for antimicrobial candidates. The materials are explored for biological activity as antimicrobial agents. Microwave reactions proceeds *via* faster rate and cleaner products with high yield.



Scheme 1 – Synthesis of Imidazolyl fluorescent dyes.

2. Experimental details

2.1. *Materials and methods*

Chemicals such as benzil, 4-methoxy aniline, ammonium acetate, 4-nitro-benzaldehyde, glacial acetic acid, ethyl acetate, hexane, 4-formylbenzoic acid, 4-aminobenzoic acid, 2-aminobenzene-1,4-dicarboxylic acid and analytical grade solvents are procured from commercially supplier, Sigma Aldrich and used as received. Melting points were recorded in a capillary tube method by Stuart Scientific apparatus and are uncorrected. ^{1}H NMR spectrum were recorded on a Jeol 400 MHz using deuterated chloroform ($CDCl_3$) and dimethyl-sulfoxide (DMSO D_6) solvent and IR spectra were recorded on a Nicolet 5700 FT-IR instrument as KBr discs. SEM (Scanning Electron Microscope) and EDAX (Energy Dispersion X-ray Analyzer) were analyzed by using Hitachi (Tabletop, Model TM 3000) Scanning Electron Microscope (SEM). Biotage microwave reactor was utilized for the synthesis of imidazole derivatives.

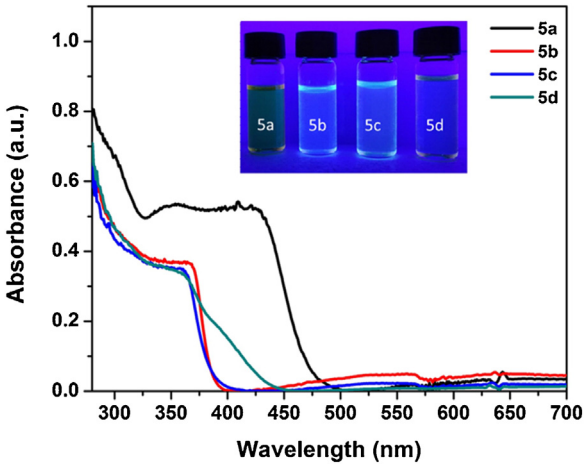
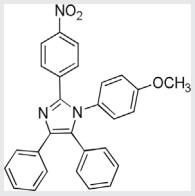
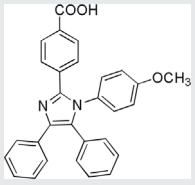
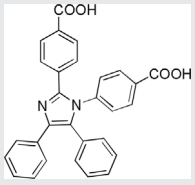
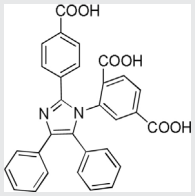


Fig. 1 – Exposure of Compounds 5a-5d under UV lamp (365 nm).

Table 1 – Molecular structure, yield and melting temperature of (5a-d).

Sl No	Structure of the compound	Name of the compound	Yield in (%)	Melting point in °C
5a		1-(4-methoxyphenyl)-2-(4chlorophenyl)-4,5-diphenyl-1*H*-imidazole	75	210–212 °C
5b		4-(1-(4-methoxyphenyl)-4,5-diphenyl-1*H*-imidazole-2-yl) phenyl carboxylic acid	70	150–152 °C
5c		4-(1-(-carboxyphenyl)-4,5-diphenyl-1*H*-imidazol-2-yl) benzoic acid	78	206–208 °C
5d		2-(2-(4-carboxyphenyl)-4,5-diphenyl-1*H*-imidazol-1-yl) benzene-1,4-dioic acid	80	210–212 °C

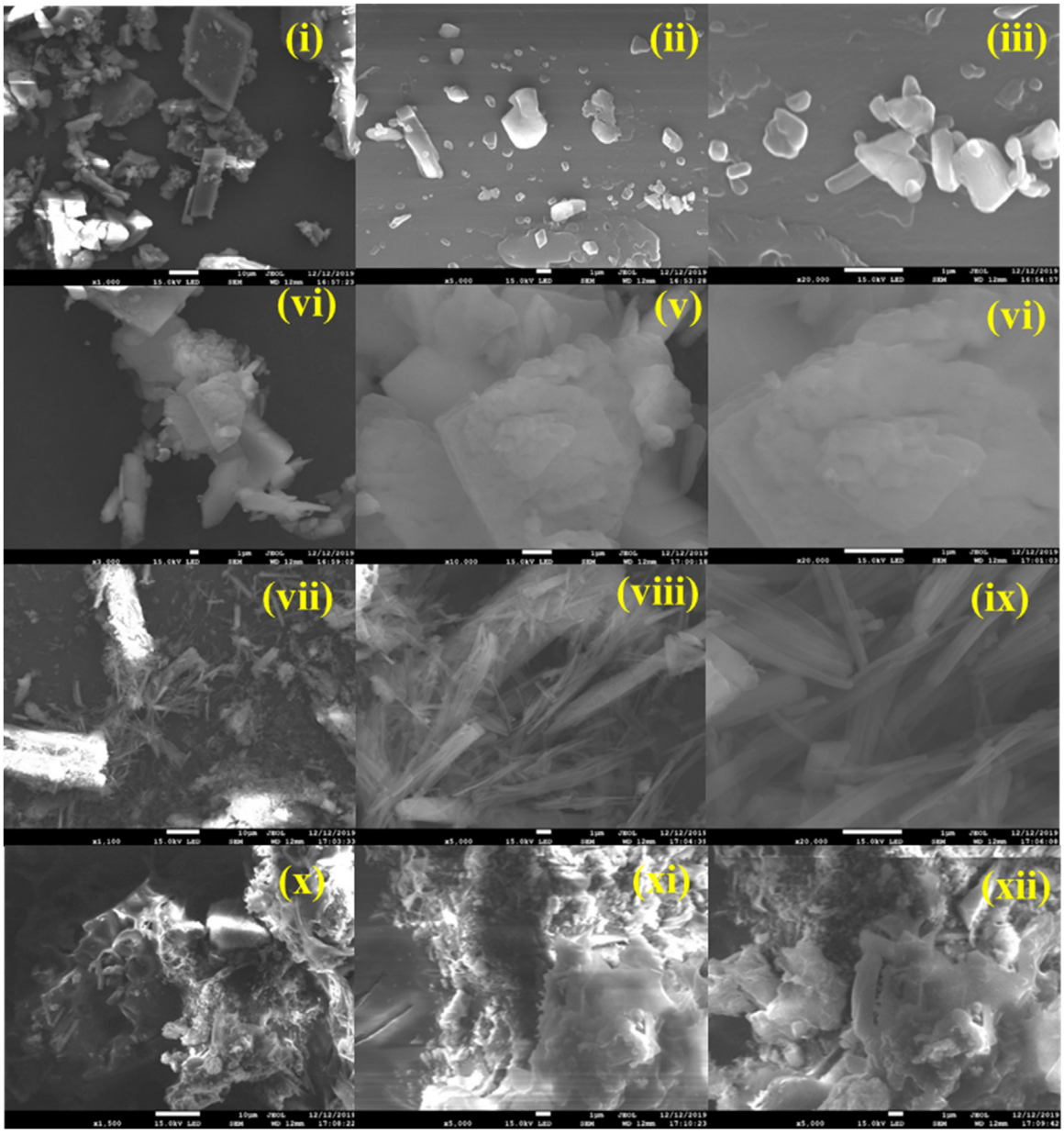


Fig. 2 – SEM image of compounds 5a (i, ii, iii), 5b (iv, v, vi), 5c (vii, viii, ix), 5d (x, xi, xii).

Table 2 – Elemental composition from EDAX Analysis of compounds 5(a–d).

Molecule	Content	Element		
		C	N	O
5a	Weight %	75.08	9.38	10.72
	Atomic %	82.35	8.83	8.82
5b	Weight %	80.75	8.22	11.03
	Atomic %	85.29	5.88	8.82
5c	Weight %	79.09	6.36	14.54
	Atomic %	82.85	5.72	11.42
5d	Weight %	74.30	5.78	19.63
	Atomic %	78.14	5.25	15.78

2.2. *Synthesis of 1-(4-methoxyphenyl)-2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazole (5a)*

Benzil (1 mmol, 0.210 g), 4-methoxy aniline (1 mmol, 0.123 g), ammonium acetate (1 mmol, 0.75 g) and 4-nitro-benzaldehyde (1 mmol, 0.150 g) were dissolved in glacial acetic acid (5 ml) were placed in a Microwave voil. Sonicate nearly 30 min then kept in a microwave for 30 min at 100 °C with power of 60 watt. The reaction was monitored by TLC [2:8 (v:v) ethyl acetate-*n*-hexane mixture]. After the completion of the reaction the reaction mixture was cooled and poured into crushed ice. The precipitated product was filtered then washed with pet ether. The crude product was then recrystallized by hot THF and ethyl acetate (2:6) to get a fine crystal of

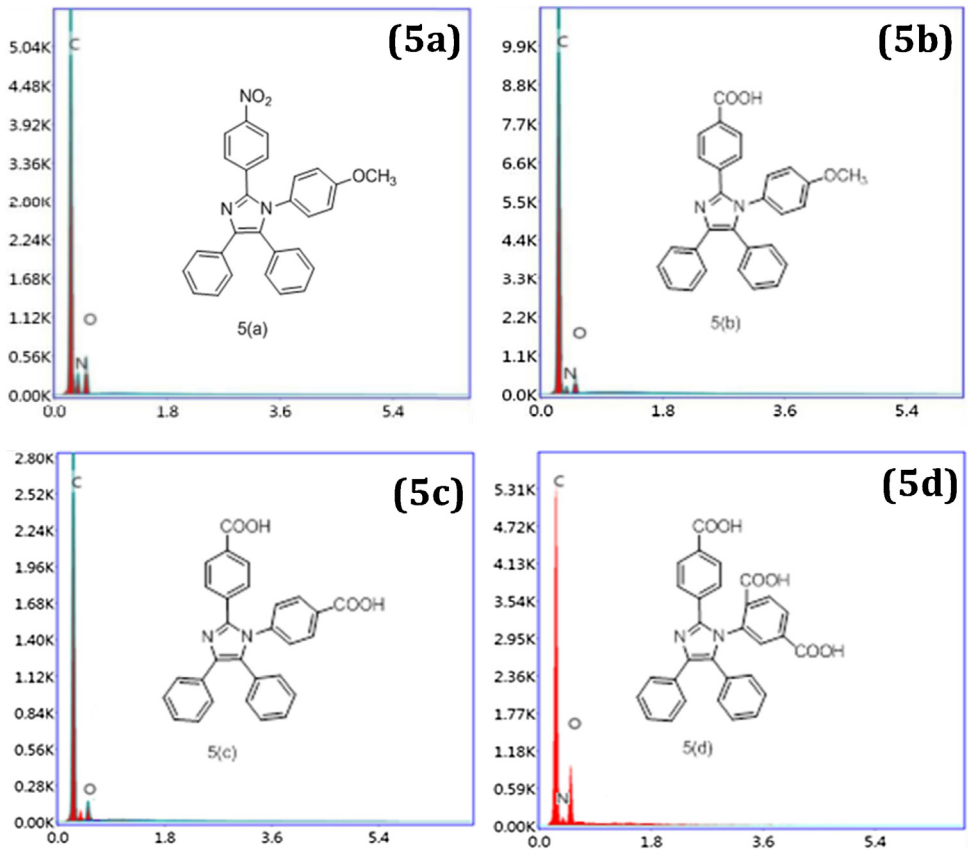


Fig. 3 – EDAX spectrum of compounds 5a-5d.

analytically pure 1-(4-methoxyphenyl)-2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazole 5a.

Appearance: greenish yellow. Yield: (75%). M.P.: 210−212 °C. IR (KBr) (cm^{-1}):1521.50 (N–O), 1577.43 (Ar C = C), 1606.87 (C = N), 2966.80 (aliphatic C–H), 3040.39 (Ar C–H), ^{1}H NMR(400 MHz, DMSO, (ppm)): δ = 3.724 (s, 3H, OCH_3), 6.875–6.914 (m, 2H, ArH), 7.166–7.208 (m, 1H, ArH), 7.236 (d, *J* = 1.6 Hz, 1H, ArH), 7.254–7.284 (m, 5H, ArH), 7.310–7.335 (m, 3H, ArH), 7.472–7.502 (m, 2H, ArH), 7.621–7.656 (m, 2H, ArH), 8.144–8.179 (m, 2H, ArH).

2.3. *Synthesis of 4-(1-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazole-2-yl) phenyl carboxylic acid (5b)*

4-methoxy aniline (1 mmol, 0.123 g), benzil (1 mmol, 0.210 g), ammonium acetate (1 mmol, 0.75 g) and 4-formylbenzoic acid (1 mmol, 0.150 g) were suspended in glacial acetic acid (5 ml) were placed in a Microwave voil. The reaction mixture was subjected to ultrasonication for 20 min, then kept in a microwave for 30 min at 100 °C with power of 60 watt. The progress of the reaction was monitored by TLC [2:8 (v:v) ethyl acetate-*n*-hexane mixture]. After the completion of the reaction, the mixture was cooled and poured into crushed ice. The reaction mixture was neutralized by aqueous sodium bicarbonate solution and the product was extracted with ethyl acetate. The crude product was then recrystallized in hot ethanol to get a fine crystal of analytically pure 4-(1-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazole-2-yl) phenyl carboxylic acid 5b.

Appearance: pale violet, Yield: (70%). M.P.:150−152 °C. IR (KBr) (cm^{-1}): 1589.21 (Ar C = C), 1606.87 (C = N), 1738.27 (Carboxylic C = O), 2922.65 (Aliphatic C–H), 2981.52 (Ar C–H), 3436.66 (Carboxylic −OH). 1H NMR (400 MHz, DMSO, (ppm)): δ = 3.710 (s, 3H, OCH_3), 6.86(d, *J* = 8.8 Hz, 2H, ArH), 7.150–7.275 (m, 7H, ArH), 7.311 (t, *J* = 6.4 Hz, 3H ArH), 7.499–7.518 (m 4H ArH), 7.83 (d *J* = 8.4 2H ArH), 12.969 (brs 1H, Carboxylic OH).

2.4. *Synthesis of 4-(1-(-carboxyphenyl)-4,5-diphenyl-1H-imidazol-2-yl) benzoic acid (5c)*

In a 250 ml round-bottom flask, benzil (1 mmol, 0.210 g), and 4-aminobenzoic acid (1 mmol, 0.137 g) ammonium acetate (1 mmol, 0.75 g) and 4-formylbenzoic acid (1 mmol, 0.1501 g), were dissolved in glacial acetic acid (5 ml) and placed in a Microwave voil. The reaction mixture was subjected to ultrasonication (20 min) then then kept in a microwave for 30 min at 100 °C with power of 60 watt. The progress of the reaction was monitored by TLC [2:8 (v:v) ethyl acetate-*n*-hexane mixture]. After the completion of the reaction, the reaction mixture was cooled to room temperature; the solution was poured into ice cold water (60 ml). Then add aqueous sodium bicarbonate to neutralize the solution. The solid separated was extracted with ethyl acetate (2 × 30 ml) and the solvent was distilled off in a rotor evaporator. The

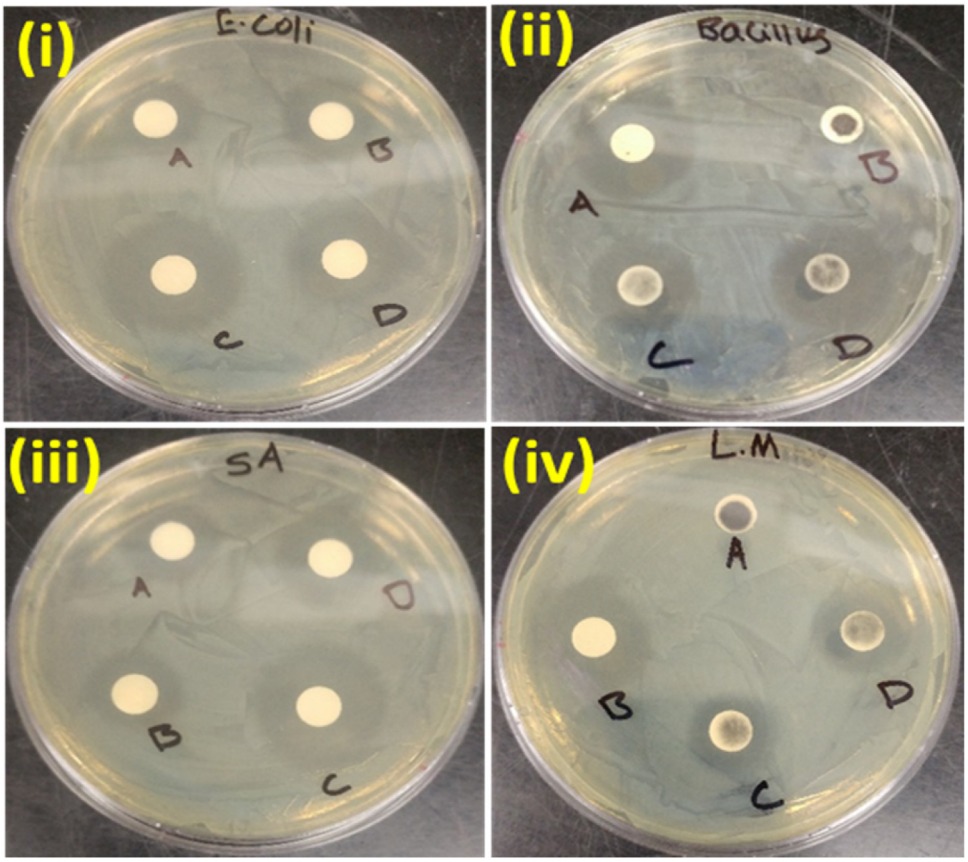


Fig. 4 – Antimicrobial activity of compounds 5(a–d) against (i) *E.coli* (ii) bacillus (iii) *S.aureus* (iv) *L.monocytogenes* (18 mm).

crude residue was purified by column chromatography using ethyl acetate-Hexane (3:7) as eluent to get a fine powder of analytically pure 4-(1-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazole-2-yl) phenyl carboxylic acid 5c.

Appearance: white solid. Yield: (78%). M.P.:206−208 °C. IR (KBr) (cm^{-1}): 1624.31 (Ar C = C), 1673.76 (C = N), 1736.15 (Carboxylic C = O), 2962.43 65 (Ar C–H), 3062.43 (Carboxylic OH). ^{1}H NMR (400 MHz, DMSO, (ppm)): δ = 6.40 (d, 4.8 Hz, 1H, ArH), 6.66-6.55 (m, 1H, ArH), 6.72–6.77 (t, 8.1 Hz, 2H, ArH),6.80–6.88 (t, 6.9 Hz, 1H ArH), 6.91–7.130 (m, 2H, ArH), 7.39–7.47 (m, 4H ArH), 7.51–7.60 (m, 2H, ArH), 7.71–7.81 (m, 2H, ArH), 7.83–7.91 (m, 1H, ArH), 7.95–8.09 (m, 1H, ArH), 8.12–8.23 (m, 1H, ArH), 10.82 (bs,1H, Carboxylic OH), 12.84 (bs,1H, Carboxylic OH).

2.5. *Synthesis of 2-(2-(4-carboxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl) benzene-1,4-dioic acid (5d)*

In a 250 ml round-bottom flask, benzil (1 mmol, 0.210 g), and 4-formylbenzoic acid (1 mmol, 0.137 g) ammonium acetate (1 mmol, 0.75 g) and 2-aminobenzene-1,4-dicarboxylic acid (1 mmol, 0.181 g), glacial acetic acid (5 ml) were placed in a Microwave voil. The reaction mixture was subjected to ultrasonication (20 min) then then kept in a microwave for 30 min at 100 °C with power of 60 watt The progress of the reaction was monitored by TLC [3:7 (v:v) ethyl acetate-*n*-hexane mixture]. After the completion of the reaction, the reaction mixture was cooled to room temperature; the solution was poured into ice cold water (60 ml). Then add aqueous sodium bicarbonate solution to neutralize the product. The solid separated was extracted with ethyl acetate (2 × 30 ml). The organic layer was dried using sodium sulphate and filtered. The solvent was distilled off in a rotor evaporator. The crude residue was purified by column chromatography using ethyl acetate-hexane (3:7) as eluent to get an analytically pure 2-(2-(4-carboxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl) benzene-1,4-dioic acid 5d.

Appearance: Pale yellow solid. Yield: (80%). M.P.: 210−212 °C IR (KBr) (cm^{-1}): 1625.87 (Ar C = C), 1673.84, (C = N), 1738.27 (Carboxylic C = O), 3051.77 (Ar C–H). 3166.97 (Carboxylic OH). ^{1}H NMR (400 MHz, DMSO, (ppm)): δ = 7.24 (d, 12 Hz, 1H, ArH), 7.38–7.41 (d, 12 Hz, 3H, ArH), 7.44 (d, 4 Hz, 5H, ArH),7.60–7.64 (m, 1H ArH), 7.90 (d, 8 Hz, 3H, ArH), 8.14 (d, 8 Hz, 1H ArH), 8.94 (d, 7.2 Hz, 3H, ArH), 10.82 (s,1H, Carboxylic OH), 11.99 (s, 2H, Carboxylic OH).

3. Results and discussions

A non-dopant deep blue fluorophores 5a–e were synthesized by a facile microwave assisted, multi component single step synthetic pathway as illustrated in Scheme-1 by adopting reported procedure [21,24] with suitable modifications. Thus, a mole equivalent of benzil (1 mmol) 1, aromatic aniline (1 mmol) 2a-d, ammonium acetate (1 mmol) 3, and aromatic

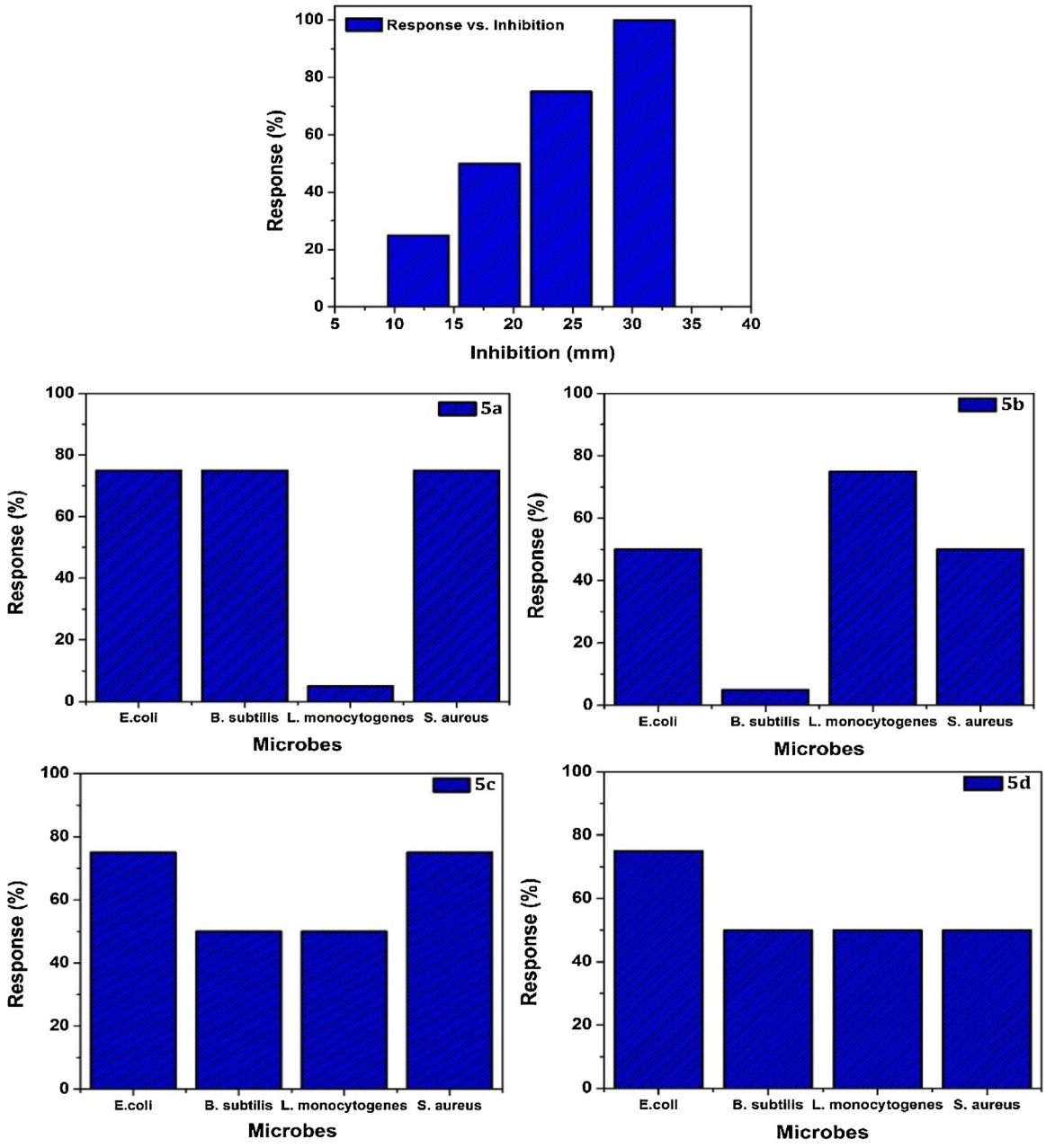


Fig. 5 – Histogram of antimicrobial data samples (reference plot and 5(a–d)).

aldehydes (1 mmol) 4a-c, were taken in acetic acid (5 ml) in a microwave vial. Reaction mixture was initially sonicated for 20 min and then treated with microwave reactor for 30 min at 100 C with 60 Watt power. The title compounds were purified by column chromatography (ethyl acetate/Hexane (3:7), and the results are given in Table 1. Chemical structures of the blue fluorophores were determined by FT-IR, ^{1}H NMR and EDAX analysis.

The infrared spectrum of 5a-d showed some characteristic Stretching bands at 1590–1635, and 1630–1725 cm^{-1} assigned to —C=C–, —C=N— and –COO- functional groups. The stretching frequency at 3040 - 3055 cm^{-1} indicates the presence of aromatic C–H stretch. All compounds except 5a, showed absorption band at 3300–3440 for carboxylic acid —OH group. Further ^{1}H NMR spectrum shows the broad peak between the 11–12 ppm due to carboxylic proton. And aromatic protons appeared in the region 6–8 ppm corresponds to 18 to 17 protons in compounds 5a-d respectively authenticate structure assigned to target molecules.

Fig. 1 shows the absorbance spectra of 5(a–d) structure after illuminating the UV light source of 365 nm for 30 min. It reveals absorption maximum in ultra-violet region for 5(b–d) samples and visible region for 5a sample, respectively. Presence of nitro group in 5a sample originates the intra molecular charge transfer process in the sample and exhibit visible light absorption from 300–450 nm. Inset of figure shows pictorial

representation of 5(a–d) samples after exposing UV light for 30 min.

SEM is one of the characteristics of material from which the exterior morphology and properties of the compounds can be determined [25]. Fig. 2, shows the typical SEM photographs of 5a–d samples respectively. In which, a heterogeneous irregular sized granular crystal like morphology with different particle sizes in micron scale was obtained for 5a and 5b samples. Micrograph images of 5a and 5b samples exhibits cutting edge having rectangular shape, irregular granular crystal-like structure, small granular like structure and stone like structure with respect to target molecules. Further, the SEM images of 5c sample displays wire like morphology and 5d sample shows agglomerated irregular shaped micro structured polymer crystals. The existence and percentage of different elements like C, N, O, and Cl was analyzed *via* EDAX spectrum (Fig. 3) and their corresponding weight percentage and atomic percentages were shown in Table 2.

The primary screening tests for microbes showed that all the compounds were found to inhibit the tested microbes. The results and observations of anti-microbial activity are summarized in Table S1 and Table S2 of supplementary information. From the table, it was observed that most of the compounds showed a high to moderate activity against the different strains of bacteria. The highest activity was shown by 5c on *E.coli* (23 mm), 5b on bacillus (18 mm), 5c on *S.aureus* (20 mm) and 5d on *L.monocytogenes* (18 mm). Lowest activity was shown by 5a on E.coli (13 mm), 5b on *S. aureus* (15 mm) and compound 5a have not shown any inhibition on *L.monocytogene* and 5b on *B.subtilis*. *L.monocytogenes* which is a very common food born pathogen and in this study, we found that three compounds inhibited its growth. These dyes can utilize in food packaging materials and sensors to detect and avoid the food pathogens growth. From the results, one can understand that the antibacterial activity strength of the synthesized dyes is mainly depending on the chemical group and position of imidazole ring. The results are in accordance with the previous reports on antimicrobial activity of different fluorescent dyes [26,27]. Antimicrobial activity is shown in Fig. 4 and their corresponding histogram of antimicrobial data were presented in Fig. 5.

4. Conclusions

In conclusion we developed, small organic imidazolyl Non-doped deep blue fluorescent dyes 5a-e *via* microwave assisted cost effective protocol. Thus, while designing desired fluorescent imidazole dyes 5a-d, a suitable auxiliary donor such as aromatic rings and —OCH_3 group on one end of the imidazolyl moiety and electron acceptors such as —NO_2 and C—OOH on other end of the compounds was achieved to get to promising fluorescent dyes for antimicrobial agents. The synthesized materials have been also screened for their antimicrobial activities indicating them as biologically active molecules.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jmrt.2020.01.011.

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