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Synthesis of 3*H*-imidazo[4,5-b] pyridine with evaluation of their anticancer and antimicrobial activity

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ABSTRACT

Microwave assisted and conventional synthetic methods of new 6-bromo-2-(substituted)-3*H*-imidazo[4,5-b]pyridine and its derivatives are described, which were obtained in reduced reaction times, higher yields, cleaner reactions than previously described methods. All the synthesized compounds were characterized, and screened for their anticancer and antimicrobial activity. Among synthesized compounds 3b and 3k shows prominent antibacterial activity and compound 3f shows both antibacterial and antifungal activity. Compounds 3h and 3j shows prominent anticancer activity against the both breast cancer cell lines, MCF-7 and BT-474. These results suggest that the imidazo[4,5-b]pyridine moiety may serve as a new promising template for synthesis of anticancer and antimicrobial agents and further study is required for evaluation of their mechanism of action.

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1. Introduction

Infections caused by multi-drug resistant bacteria are of major health concern worldwide. The imidazole and benzimidazole nucleus are important building blocks in the drug discovery. The imidazole ring system is considered to be one of the most imperative heterocyclic substructures found in a large number of natural products and pharmacologically active compounds. An examination of literature revealed that imidazole and their analogues usually possess diverse biological activities like antimicrobial, antioxidant, antihemolytic, cytotoxic [1] and antimycobacterial [2]. Almost all of the major classes of antibiotics have encountered resistance in clinical applications [3]. Because of the versatile core contained in several substances of imidazole derivatives are possess a broad spectrum of pharmacological activities [4,5]. Recently imidazole and its derivatives has been the subject of extensive study of the antimicrobial activity for their potential as effective therapeutic agents. Based on several literature surveys, imidazole derivatives show a range of pharmacological activities, such as antimicrobial, anti-tubercular, antiviral [6-11], antioxidant and antifungal [12], antidepressant [13], anti-inflammatory and analgesic [14], anti-tuberculosis [15] anticancer [16,17]. The imidazole scaffold is present in many natural products and is a bioactive substance in human metabolism [18]. There are many clinical drugs being used in the different therapeutic areas based on the imidazole structure, such as antihistaminic (cimetidine), anti-cancer (dacarbazine), anti-parasitic (metronidazole), and antihypertensive (losartan) (Figure 1).

Many heterocyclic cores have played significant role in the pharmacological activities, like pyridine core, the high therapeutic properties of pyridine related drugs have encouraged medicinal chemists to the synthesized large number of chemotherapeutic agents. The medicinal properties of pyridine include a variety of pharmacological applications, such as antibacterial [19], antiparacite [20], antitumor [21], anticancer [22,23], antiviral [24] and anti-inflammatory [25]. In addition, they can act as antagonists of various biological receptors [26]. Various methods for the synthesis of multi-substituted imidazole derivatives involving different catalysts including $AlCl_3$, $FeCl_3$, $Yb(OTf)_3$, $NdCl_3$, $LaCl_3$ [27], $ZrOCl_2.8H_2O$ [28], $BF_3.SiO_2$, zeolite [29], NaH_2PO_4 [30], cyclic phosphoric acid [31] and SiO_2 [32] etc.

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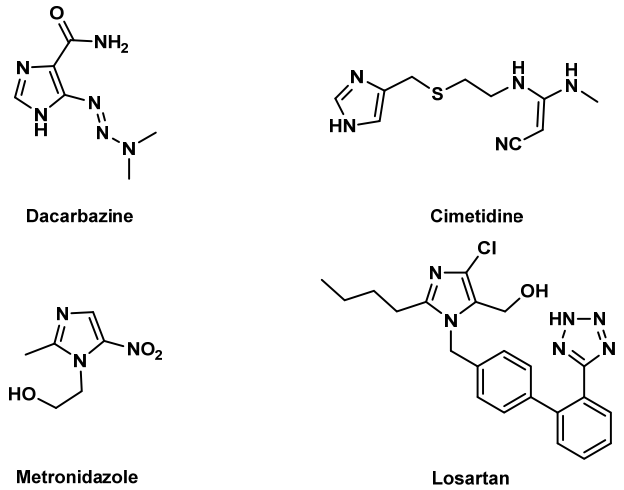


Figure 1. Examples of significant imidazole containing pharmaceuticals.

The application of microwave irradiation is the use of catalysts or mineral supported reagents, under solvent-free conditions, enables organic reactions to occur expeditiously in ambient pressure, thus providing unique chemical processes with special attributes such as enhanced reaction rates and higher product yields [33,34].

2. Experimental

2.1. Materials and instrumentations

5-Bromopyridine-2, 3-diamine, substituted aldehydes, *N*,*N*-dimethylformamide, glacial acetic acid and various solvents were commercially available. The major chemicals were purchased from Sigma Aldrich and Avra labs. Reaction progress was monitored by TLC on silica gel precoated F_{254} Merck plates. Developed plates were examined with ultraviolet lamps (254 nm). IR spectra were recorded on a FT-IR (Bruker). Melting points were recorded on SRS Optimelt, melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a 400 MHz Bruker spectrometer and ^{13}C NMR spectra were recorded on a 100 MHz Bruker spectrometer are reported as parts per million (ppm) downfield from a tetramethylsilane internal standard. The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of water mass spectrometer. Microwave reactions were carried out in MicroSYNTH Lab station of Ethusi Milestone.

2.2. General procedure for the synthesis of compounds (3a-l)

2.2.1. Microwave-assisted synthesis of of 6-bromo-2-(substituted) -3H-imidazo [4,5-b] pyridine, Method A

An equimolar amounts of 5-bromopyridine-2,3-diamine (**1**) (1 mmol), substituted aldehydes (**2a-l**) (1 mmol) was added in DMF. This mixture was taken in a 100 mL round bottom flask subjected to MW irradiation (900 W), at 110 °C temperature for 5-6 min. The completion of the reaction progress was monitored by using TLC (10%, ethyl acetate: *n*-hexane). The product obtained was poured into water and ethyl acetate (2:8, *v*:*v*) (3×10 mL). The combined solvent extracts were concentrated *in vacuo*. The compounds were recrystallized from ethanol to give a pure product (**3a-l**).

2.2.2. Conventional synthesis of 6-bromo-2-(substituted) -3H-imidazo [4,5-b] pyridine, Method B

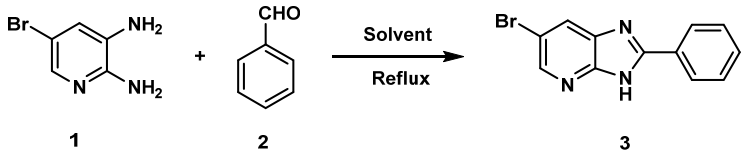
An equimolar amounts of 5-bromopyridine-2, 3-diamine (**1**) (1 mmol), substituted aldehydes (**2a-l**) (1 mmol) were added in DMF. This mixture was taken in a 100 mL round bottom flask and stirring on reflux up to 3-4 h. The completion of the reaction progress was monitored by using TLC (10%, ethyl acetate: *n*-hexane). The product obtained was poured into water and ethyl acetate (2:8, *v*:*v*) (3×10 mL). The combined solvent extracts were concentrated *in vacuo*. The compounds were recrystallized from ethanol to give a pure product (**3a-l**) (Scheme 1).

6-Bromo-2-phenyl-3H-imidazo[4,5-b]pyridine (**3a**): Color: Yellow solid. Yield: 99%. M.p.: 165-167 °C. FT-IR (KBr, ν, cm^{-1}): 3059 (NH), 2843 (aromatic CH), 1569 (C=N), 1447 (C=C), 702 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.60-6.95 (m, 5H, H-Aromatic), 7.62 (s, 1H, pyridine H), 9.40 (s, 1H, pyridine H), 10.10 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 119.2, 127.5, 129.3, 131.2, 131.8, 134.7, 138.8, 148.4, 150.2, 161.8. MS (EI, *m/z* (%)): 276.18 (M+2).

4-(6-Bromo-3H-imidazo[4,5-b]pyridin-2-yl)phenol (**3b**): Color: Yellow solid. Yield: 96%. M.p.: 192-194 °C. FT-IR (KBr, ν, cm^{-1}): 3375 (OH), 3119 (NH), 3013 (aromatic CH), 1612 (C=N), 1468 (C=C), 825 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 5.50 (s, 1H, OH), 6.85-6.87 (d, 2H, H-Aromatic), 6.96-6.98 (d, 2H, H-Aromatic), 8.45 (s, 1H, pyridine), 9.40 (s, 1H, pyridine), 10.10 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 116.4, 119.1, 127.4, 130.5, 131.6, 138.9, 148.2, 150.3, 158.8, 161.1. MS (EI, *m/z* (%)): 290.25.

6-Bromo-2-(2,4-dichlorophenyl)-3H-imidazo[4,5-b]pyridine (**3c**): Color: Yellow solid. Yield: 95%. M.p.: 214-216 °C. FT-IR (KBr, ν, cm^{-1}): 3027 (NH), 3008 (aromatic CH), 1578 (C=N), 1461 (C=C), 790 (C-Cl), 688 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.70-8.00 (m, 3H, H-Aromatic), 8.10 (s, 1H, pyridine), 8.20 (s, 1H, pyridine), 8.80 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 119.0, 127.5, 130.6, 130.9, 131.7, 133.5, 133.7, 135.5, 136.5, 148.1, 150.4, 158.8. MS (EI, *m/z* (%)): 343.00.

6-Bromo-2-(4-chlorophenyl)-3H-imidazo[4, 5-b]pyridine (**3d**): Color: Yellow solid. Yield: 96%. M.p.: 137-139 °C. FT-IR (KBr, ν, cm^{-1}): 3137 (NH), 1653 (C=N), 1464 (C=C), 760 (C-Cl), 631 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.50-7.52 (d, 2H, H-Aromatic), 7.68-7.70 (d, 2H, H-Aromatic), 7.95 (s, 1H, pyridine), 8.15 (s, 1H, pyridine), 8.80 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 119.0, 128.5, 129.6, 131.8, 132.5, 134.7, 138.5, 148.1, 150.4, 161.7. MS (EI, *m/z* (%)): 308.50.



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Reaction condition: 5-bromopyridine-2,3-diamine (**1**) (1 mmol), benzaldehyde (**2**) (1 mmol), solvent 1 mL, reflux 3-12 h.

Scheme 1

6-Bromo-2-(2-chlorophenyl)-3H-imidazo[4, 5-b]pyridine (**3e**): Color: Yellow solid. Yield: 94%. M.p.: 157-159 °C. FT-IR (KBr, ν, cm^{-1}): 3065 (NH), 1653 (C=N), 1457 (C=C), 702 (C-Cl), 637 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.60-7.86 (m, 4H, H-Aromatic), 8.20 (s, 1H, pyridine), 8.50 (s, 1H, pyridine), 9.15 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 119.1, 127.5, 128.4, 129.5, 130.1, 131.7, 132.4, 134.7, 138.5, 147.5, 148.0, 150.5. MS (EI, *m/z* (%)): 308.85.

6-Bromo-2-(4-methoxyphenyl)-3H-imidazo[4, 5-b]pyridine (**3f**): Color: Yellow solid. Yield: 95%. M.p.: 191-193 °C. FT-IR (KBr, ν, cm^{-1}): 3198 (NH), 3006 (aromatic CH), 1553 (C=N), 1461 (C=C), 634 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.75 (s, 3H, OCH_3), 7.05-7.26 (m, 4H, H-Aromatic), 8.54 (s, 1H, pyridine), 9.14 (s, 1H, pyridine), 10.76 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 55.9, 114.8, 119.1, 127.4, 130.3, 131.9, 138.8, 148.0, 150.5, 160.6, 161.5. MS (EI, *m/z* (%)): 304.23.

6-Bromo-2-(2,4-dimethoxyphenyl)-3H-imidazo[4, 5-b]pyridine (**3g**): Color: Yellow solid. Yield: 93%. M.p.: 196-198 °C. FT-IR (KBr, ν, cm^{-1}): 3199 (NH), 3016 (aromatic CH), 1563 (C=N), 1451 (C=C), 638 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.76 (s, 6H, OCH_3), 7.15-7.46 (m, 3H, H-Aromatic), 8.52 (s, 1H, pyridine), 9.11 (s, 1H, pyridine), 10.71 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 55.8, 56.8, 98.8, 107.6, 110.1, 119.3, 129.4, 131.9, 138.8, 148.0, 150.4, 151.6, 158.3, 161.6. MS (EI, *m/z* (%)): 334.25.

6-Bromo-2-(4-fluorophenyl)-3H-imidazo[4, 5-b]pyridine (**3h**): Color: Yellow solid. Yield: 93%. M.p.: 270-272 °C. FT-IR (KBr, ν, cm^{-1}): 3219 (NH), 3051 (aromatic CH), 1590 (C=N), 1463 (C=C), 904 (C-F), 640 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.43-7.78 (m, 4H, H-Aromatic), 8.51 (s, 1H, pyridine), 9.10 (s, 1H, pyridine), 10.75 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 116.0, 119.5, 129.6, 130.8, 131.5, 138.5, 148.1, 150.4, 161.7, 162.9. MS (EI, *m/z* (%)): 292.11.

6-Bromo-2-(3-fluorophenyl)-3H-imidazo[4, 5-b]pyridine (**3i**): Color: Yellow solid. Yield: 94%. M.p.:147-149 °C. FT-IR (KBr, ν, cm^{-1}): 3218 (NH), 3053 (aromatic CH), 1577 (C=N), 1457 (C=C), 965 (C-F), 674 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.33-7.79 (m, 4H, H-Aromatic), 8.55 (s, 1H, pyridine), 9.16 (s, 1H, pyridine), 10.78 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 115.5, 116.1, 119.5, 123.2, 127.8, 131.5, 132.4, 138.7, 148.2, 150.6, 161.7, 162.5. MS (EI, *m/z* (%)): 292.10.

6-Bromo-2-(4-nitrophenyl)-3H-imidazo[4, 5-b]pyridine (**3j**): Color: Yellow solid. Yield: 94%. M.p.: 185-187 °C. FT-IR (KBr, ν, cm^{-1}): 3228 (NH), 3033 (aromatic CH), 1513 (C=N), 1469 (C=C), 1339 (NO_2), 686 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.03 (m, 4H, H-Aromatic), 8.58 (s, 1H, pyridine), 9.06 (s, 1H, pyridine), 10.79 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 119.5, 124.2, 127.2, 131.5, 138.7, 140.3, 147.6, 148.2, 150.6, 161.7. MS (EI, *m/z* (%)): 319.20.

4-(6-Bromo-3H-imidazo[4, 5-b]pyridin-2-yl)benzonitrile (**3k**): Color: Yellow solid. Yield: 92%. M.p.: 260-262 °C. FT-IR (KBr, ν, cm^{-1}): 3226 (NH), 3022 (aromatic CH), 2224 (CN), 1569 (C=N), 1464 (C=C), 624 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.53-7.79 (m, 4H, H-Aromatic), 8.54 (s, 1H, pyridine), 9.09 (s, 1H, pyridine), 10.76 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 112.3, 118.6, 119.2, 127.2, 131.5, 132.4, 138.7, 139.3, 148.2, 150.6, 161.7. MS (EI, *m/z* (%)): 299.10.

6-Bromo-2-(thiophen-2-yl)-3H-imidazo[4, 5-b]pyridine (**3l**): Color: Yellow solid. Yield: 96%. M.p.: 160-162 °C. FT-IR (KBr, ν, cm^{-1}): 3224 (NH), 3065 (aromatic CH), 1594 (C=N), 1457 (C=C), 702 (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.35-7.72 (m, 3H, H-Aromatic), 8.53 (s, 1H, pyridine), 9.12 (s, 1H, pyridine), 10.86 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 119.2, 128.2, 129.1, 131.6, 131.8, 132.4, 138.7, 143.2, 148.2, 150.6. MS (EI, *m/z* (%)): 280.20.

2.3. Biological evaluation

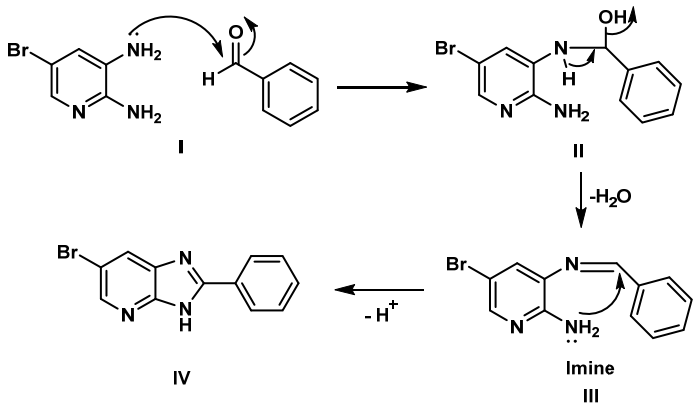
2.3.1. Antibacterial activity

All the synthesized compounds (**3a-l**) were screened for their *in vitro* antibacterial activity; three Gram positive bacteria; *Bacillus subtilis* (NCIM-2063), *Enterococcus faecalis* (NCIM-5443) and *Staphylococcus aureus* (NCIM-2901), three Gram negative bacteria; *Escherichia coli* (NCIM-2256), *Pseudomonas aeruginosa* (NCIM-2037) and *Salmonella typhimurium* (NCIM-2501). The organisms were obtained from the National Chemical Laboratory, Pune, MS, India. The antibacterial assay was carried out by a microdilution method [35-37] in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. The inocula were prepared daily and stored at +4 °C until use. The dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculums. All experiments were performed in duplicate and repeated three times.

2.3.2. Antifungal activity

All the synthesized compounds (**3a-l**) were screened for their *in vitro* antifungal activity. Six fungal strains; *Candida albicans* (NCIM-3471), *Aspergillus flavus* (NCIM-539), *Aspergillus oryzae* (NCIM-570), *Aspergillus Niger* (NCIM-1196), *Penicillium chrysogenum* (NCIM-707) and *Fusarium oxysporum* (NCIM-1282). The organisms were obtained from the National Chemical Laboratory, Pune, MS, India. The micromycetes were maintained on malt agar and the cultures stored at +4 °C and sub-cultured once a month. In order to investigate the antifungal activity of the compounds, a modified microdilution technique was used [38]. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (*v*:*v*). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 µL per well. The inocula were stored at +4 °C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum.

The Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in 5% DMSO solution containing 0.1% Tween 80 (*v*:*v*) (1 mg/mL) and added in broth Malt medium with inoculum.



Scheme 2

The microplates were incubated at Rotary shaker (160 rpm) for 72 h at 28 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial sub-cultivation of a 2 µL of tested compounds dissolved in medium and inoculated for 72 h, into microtiter plates containing 100 µL of broth per well and further incubation 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. The DMSO was used as a negative control, commercial fungicides; fluconazole and miconazole were used as positive controls (1-3000 µg/mL). All experiments were performed in duplicate and repeated three times.

2.3.3. Microdilution test

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtiter plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. The compounds to be investigated were dissolved in 5% DMSO solution containing 0.1% Tween 80 (*v:v*) (1 mg/mL) and added in broth LB medium (100 µL) with bacterial inoculum (1.0×10^4 CFU per well) to achieve the wanted concentrations. The micro-plates were incubated at Rotary shaker (160 rpm) for 24 h at 48 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 µL into microtiter plates containing 100 µL of broth per well and further incubation for 24 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 and compared with a blank and the positive control. The ciprofloxacin and ampicillin were used as a positive control (1 mg/mL DMSO). The DMSO was used as a negative control. All experiments were performed in duplicate and repeated three times.

2.3.4. Anticancer activity

All the synthesized compounds were also tested for their in vitro anticancer activity against two human breast cancer cell lines MCF-7 and BT-474. The anticancer activity test is performed according to the procedure developed by the National Cancer Institute (NCI, USA) in the '*In vitro* Anticancer Drug Discovery Screen' that uses the protein-binding dye Sulforhodamine B (SRB) to assess cell growth [39,40]. Briefly, cells are grown in 96-well plates in suspension and then were exposed for 48 hours to four serial concentrations of 10^{-7} Molar (M), 10^{-6} M, 10^{-5} M and 10^{-4} M of each compound. Following this, cells were fixed and stained with protein binding SRB stain. Excess stain is washed and bound stain was solubilized, and the absorbance was measured at 492 nm in a plate reader. Concentration of the compounds that inhibited 50% of the net cell growth, growth inhibition of 50% (GI_{50}), was calculated from the dose response curve obtained for each test compound and cell line. GI_{50} values were presented in micro molar (µM) concentration. Adriamycin (Doxorubicin) was used as positive control for the comparison of cytotoxicity of synthesized compounds. Assays were performed in triplicate on three independent experiments and their mean values are taken as a final reading.

3. Results and discussion

3.1. Chemistry

We have developed the protocol for the synthesis of 6-bromo-2-phenyl-3H-imidazo[4,5-b]pyridine (**3**) (Scheme 1) by condensation between 5-bromopyridine-2,3-diamine and benzaldehyde. In this reaction, various solvents were selected as a model reaction to optimize the reaction conditions. In terms of the effect of solvent on the condensation reaction, DMF was found to be the best solvent for the reaction (Table 1, entry 4); other solvents, including ethanol, methanol, benzene, toluene and tetrahydrofuran were less efficient (Table 1, entries 1, 2, 3, 5 and 6). Ethanol, methanol, benzene, toluene and tetrahydrofuran gave the corresponding product yield 30, 45, 50, 40 and 45%, respectively, which were the worst among these solvents. Nevertheless, all of these yields were generally low before further optimizations. All the reactions were carried out with equimolar amounts of each compound in 1 mL of solvent. Among these reactions same amounts of the solvent, DMF turned out to be the best choice with yields of 98% (Table 1, entries 4). We would like to mention here that DMF as a solvent was the best choice with a yield of 98% and less time required for the completion of the reaction (Table 1, entry 4). Thus, we decided to carry out the reactions in DMF solvent.

The plausible reaction mechanism is shown in Scheme 2. The initial reaction of 5-bromopyridine-2,3-diamine with benzaldehyde produces imine, which at the reflux temperature is dehydrated or loss of water. Then the cyclise the ring, product are formed by the loss of one proton.

In continuation of our work [41-48], on the synthesis of bioactive compounds, we have synthesized some imidazole analogues. In view of the facts mentioned above, imidazole derivatives were synthesized.

Table 1. Screening of solvents, reaction time, and yield for the synthesis (**3**) [a].

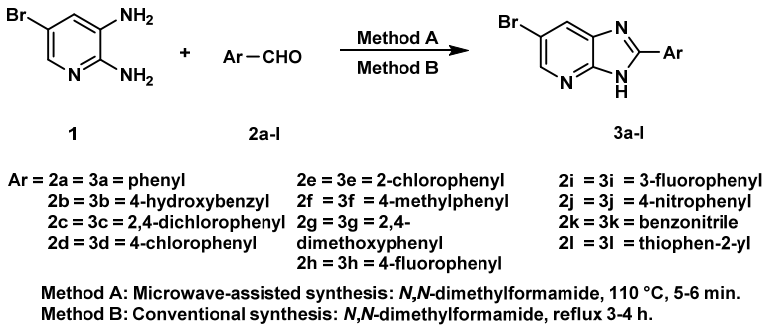
Entry	Solvent	Time (h)	Yield [b] (%)
1	Ethanol	11	30
2	Methanol	12	45
3	Benzene	10	50
4	DMF	3	98
5	Toluene	7	40
6	Tetrahydrofuran	11	45

[a] All the reaction was carried out in equimolar amounts of each compound in 1 mL of solvent.
[b] Isolated yield.

Table 2. Synthesis of 6-bromo-2-(substituted)-3*H*-imidazo [4, 5-b] pyridine (**3a-l**) [a].

Compound	Aldehydes (2a-l)	Time (min)		Yield [b] %		M.p (°C)
		MW [c] (min)	Conv. [d] (h)	MW [c]	Conv. [d]	
3a	Benzaldehyde	5	3	99	98	165-167
3b	4-Hydroxybenzaldehyde	5	3	96	88	192-194
3c	2,4-Dichlorobenzaldehyde	6	4	95	86	214-216
3d	4-Chlorobenzaldehyde	6	3	96	90	137-139
3e	2-Chlorobenzaldehyde	6	4	94	90	157-159
3f	4-Methoxybenzaldehyde	5	4	95	86	191-193
3g	2,4-Dimethoxybenzaldehyde	5	3	93	85	196-198
3h	4-Fluorobenzaldehyde	6	4	93	90	270-272
3i	3-Fluorobenzaldehyde	6	4	94	86	147-149
3j	4-Nitrobenzaldehyde	5	3	94	88	185-187
3k	4-Formylbenzonitrile	5	3	92	82	260-262
3l	Thiophene-2-carbaldehyde	6	4	96	90	160-162

[a] Reaction condition (**3a-l**), Method A: Microwave-assisted synthesis: *N*,*N*-dimethylformamide, 110 °C, 5-6 min; Method B: Conventional synthesis: *N*,*N*-dimethylformamide, reflux 3-4 h.
[b] Isolated yields.
[c] Microwave.
[d] Conventional.



Scheme 3

With this in mind, we initiated a program to synthesize a series of new 6-bromo-2-(substitu-ted)-3*H*-imidazo[4,5-b]pyridine derivatives, which have different pharmacological active groups, which can exhibit anticancer and antimicrobial activity. We have successfully synthesized series of new 6-bromo-2-(substituted)-3*H*-imidazo[4,5-b]pyridine (**3a-l**) (Scheme 3) by using *N*,*N*-dimethylformamide (DMF) as a solvent with glacial acetic acid in catalytic amounts, under microwave-assisted technique as well as conventional method. All the synthesized compounds are tested for their *in vitro* antimicrobial activities against selected bacterial and fungal strains as well as for anticancer against MCF-7 and BT-474 human breast cancer cell line.

Our current investigation describes the convenient synthesis of new 6-bromo-2-(substituted)-3*H*-imidazo[4,5-b]pyridine (**3a-l**) (Scheme 3) using DMF as a solvent under microwave assisted as well as conventional synthesis with high yield in a short time period. This method is unique, rapid and convenient for the synthesis 3*H*-imidazo[4,5-b]pyridine derivatives. We herein report, the synthesis and screening of anticancer and antimicrobial activity of this series. Microwave used in the study was MicroSYNTH Lab station of Ethusi Milestone with temperature control (Scheme 3, method A). A one-pot, microwave assisted as well as conventional synthesis by using a 5-bromopyridine-2,3-diamine, substituted aldehydes and DMF as a solvent to give the compounds (**3a-l**) (Table 2). These compounds were characterized on the basis of spectral analysis. The IR spectrum of representative compound 6-bromo-2-phenyl-3*H*-imidazo[4,5-b]pyridine (**3a**), IR absorption bands in the wave numbers show 3065 cm^{-1} that is due to a secondary amine group, 702 cm^{-1} that is due to a bromine atom. The mass spectrum revealed a molecular ion peak at *m/z* was 276.18 (M+2) corresponding to a molecular formula $C_{12}H_8BrN_3$. The ^1H NMR spectrum of the compound **3a** has been shown multiplate of benzyl proton in the range of δ ppm 6.60-6.95 (m, 5H) and 7.62 (s, 1H, pyridine H), 9.40 (s, 1H, pyridine H), 10.10 (s, 1H, NH). The synthesized compounds were characterized on the basis of IR, ^1H NMR, ^{13}C NMR, Mass spectral analysis.

3.2. Biological evaluation

3.2.1. Antimicrobial activity

From the antimicrobial data it is clearly observed that many of the synthesized compounds were shows prominent antimicrobial activity (Table 3 and 4). Antimicrobial data indicate that among the twelve synthesis compounds of present series many shows promising good to moderate level of antimicrobial activity. Some compounds were narrow spectrum, active against only one fungal or bacterial strain while some of them were found to be broad spectrum molecules, active against both one and more fungal and bacterial strains.

Table 3. Antibacterial activities of title compounds (**3a-l**).

Compounds		MIC Values (µg/mL) [a]					
		B. subtilis	*S. aureus*	*E. coli*	*E. faecalis*	*P. aeruginosa*	*S. typhimurium*
3a	MIC	85	95	80	80	90	75
	MBC	100	115	100	95	110	95
3b	MIC	70	55	3.25	60	75	70
	MBC	92	85	55	90	95	100
3c	MIC	30	60	55	70	85	90
	MBC	60	85	70	95	100	115
3d	MIC	15	55	10	20	10	60
	MBC	45	80	35	55	30	100
3e	MIC	20	65	15	70	80	75
	MBC	60	90	30	100	110	100
3f	MIC	25	95	20	20	90	25
	MBC	60	115	70	45	120	60
3g	MIC	90	100	75	100	95	85
	MBC	110	120	95	120	110	100
3h	MIC	55	70	80	65	75	60
	MBC	70	100	110	90	95	100
3i	MIC	80	70	90	95	85	90
	MBC	100	90	120	120	100	115
3j	MIC	70	50	60	65	70	65
	MBC	95	85	90	90	95	100
3k	MIC	6.0	60	75	95	80	70
	MBC	50	85	95	90	100	110
3l	MIC	90	100	75	80	85	90
	MBC	120	120	100	115	115	110
Ciprofloxacin	MIC	6.25	6.25	4.0	6.25	6.25	4.0
	MBC	20	20	20	20	20	20
Ampicillin	MIC	12.5	12.5	12.5	12.5	12.5	12.5
	MBC	25	25	25	25	25	25

[a] Values are the average of three readings.

Table 4. Antifungal activity of title compounds (**3a-l**).

Compounds		MIC Values (µg/mL) [a]					
		A. oryzae	*P. chrysogenum*	*F. oxysporum*	*C. albicans*	*A. flavus*	*A. niger*
3a	MIC	95	80	85	95	100	90
	MFC	130	105	110	100	115	120
3b	MIC	65	85	70	60	85	90
	MFC	90	115	100	95	100	130
3c	MIC	75	100	90	60	90	80
	MFC	95	130	125	100	115	95
3d	MIC	90	70	85	70	80	95
	MFC	115	100	115	105	110	115
3e	MIC	85	90	100	20	85	90
	MFC	100	115	130	60	110	120
3f	MIC	30	100	95	90	30	35
	MFC	60	125	125	110	70	65
3g	MIC	100	80	85	90	85	90
	MFC	120	115	105	130	100	115
3h	MIC	95	85	90	65	85	90
	MFC	115	120	105	95	100	125
3i	MIC	95	80	90	85	95	100
	MFC	130	100	115	100	130	115
3j	MIC	95	90	100	70	80	75
	MFC	120	105	130	95	100	95
3k	MIC	70	65	75	70	90	80
	MFC	115	90	100	95	130	125
3l	MIC	95	90	100	80	85	90
	MFC	115	130	125	105	100	120
Fluconazole	MIC	6.25	10	6.25	10	6.25	6.25
	MFC	55	50	45	40	50	40
Miconazole	MIC	3.25	3.25	3.25	3.25	3.25	3.25
	MFC	40	35	45	40	35	40

[a] Values are the average of three readings.

Among the series compounds **3b** and **3k** were found to be the narrow spectrum molecule as they were specifically active against the bacterium *E. coli* and *B. subtilis*, respectively. They are also most active molecules among the series, more potent than standard antibacterial drugs Ciprofloxacin and Ampicillin, with a MIC of 3.25 µg/mL for compound **3b** and 6.0 µg/mL for compound **3k**. On the other hand, compounds **3f**, **3f** and **3c** are found to be quite broad spectrum molecules as they are active against one or more bacterial as well as fungal strains. Compounds **3f** were found active against the majority of testing strains in the present studies**,** bacteria *B. subtilis* (MIC 25) and *E. coli* (MIC 20), *E. faecalis* and *S. typhimurium*, and fungus *A. flavus* (MIC 30) and *A. niger* (MIC 35) and *A. oryzae* (MIC 30). While the compound **3e** is active against the fungus *C. albicans* (MIC 20), in addition to bacterial strains *B. subtilis* (MIC 20) and *E. coli* (MIC 15). The compound **3c** has the same activity profile of the compound **3e** but with somewhat high MIC values. However, the compound **3d** is bacterial specific, active agaist the four bacterial strains, *B. subtilis* (MIC 15), *E. faecalis* (MIC 20), *E. Coli* (MIC 10) and *P. aeruginosa* (MIC 10). Remaining compounds of the series **3a**, **3g**, **3i** and **3l** are found to be biologically inactive in nature owing to their high MIC value ranges from 75 to 100 µg/mL and their neutral nature towards the both cancer cell lines.

Table 5. Anticancer activity of the compounds (**3a-l**).

Compounds	(IC_{50}) [a] µMolar [b]		Compounds	(IC_{50}) [a] µMolar [b]	
	MCF-7 [c]	BT-474 [d]		MCF-7 [c]	BT-474 [d]
3a	68.8	56.8	3g	88.4	68.5
3b	65.7	41.0	3h	1.7	0.9
3c	64.8	48.4	3i	82.2	87.1
3d	59.5	56.2	3j	1.3	1.1
3e	59.7	57.6	3k	75.7	66.5
3f	80.5	48.6	3l	81.1	81.2
Adriamycin [e]	<0.1				

[a] GI50 (Growth inhibition of 50) : Concentration of drug that decreases the growth of the cells by 50% compared to non-treated control cell.
[b] Values are the average of three readings.
[c] MCF-7: Human Breast cancer cell line.
[d] BT474: Human Breast cancer cell line.
[e] Adriamycin : Positive control compound.

3.2.2. Anticancer activity

The result of this study (Table 5) indicates that compound **3h** and **3j** shows prominent anticancer activity against both cell lines, having Growth inhibition of 50 (GI_{50}) values of 0.9 to 1.7 µM. All experiments were performed in duplicate and repeated three times. The structure activity relationship of the series can be explained as,

Effect of phenyl ring: Presence of only phenyl ring, without any substitution, at C2 position on imidazole ring (**3a**), in 6-bromo-2-(substituted)-3H-imidazo[4,5-b]pyridine moiety is inactive in nature, do not show any antimicrobial and anticancer activity. It indicates that, to attain the antimicrobial activity, substituted groups may present on the phenyl ring.

Effect of hydroxyl group: Substitution of the hydroxyl group at the para position on the phenyl ring (**3b**), in 6-bromo-2-(substituted) -3H-imidazo [4,5-b] pyridine moiety, make the molecule narrow spectrum and active only against bacterium *E. coli.*

The effect of nitrile group: New 6-bromo-2-(substituted)-3H-imidazo[4,5-b]pyridine moiety containing nitrile groups at the para position on the phenyl ring (**3k**) shows strain specific activity, active only against bacterium *B. subtilis.*

Effect of chloro group: Introduction of the *chloro* group in the *para* position (**3d**) on the phenyl ring make a molecule bacterial specific; active towards *B. subtilis E. faecalis, P. aeruginosa* and *E. coli.* On the other hand, changing the position of this group from *para* to *ortho* (**3e**) on the phenyl ring make the molecule also active towards the fungus *C. albicans* along with its antibacterial properties. This indicates that changing the position of the chloro group on phenyl ring, from *para* to *ortho*, also changes its antimicrobial activity profile, and this change is favorable for the development of antimicrobial active molecules by incorporating bromo-2-(substituted)-3H-imidazo[4,5-b]pyridine moiety. However, interestingly simultaneous presents of the chloro group in the *ortho* and *para* position on the phenyl ring (**3c**) make the molecule inactive in nature. This may happen due to increase the size of molecules as well as the steric crowding effect imposed by two chloro groups, inhibiting the binding of molecules to its target in biological system.

The effect of methoxy group: Introduction of methoxy group on the phenyl ring at the *para* position (**3f**) makes the molecule broad spectrum; active against most of the bacterial and fungal strains tested. On the other hand simultaneously substitution of methoxy group at *ortho* and *para* position (**3g**) result in inactivation of the molecule against all bacteria and fungus. It may be due to increase the electron donation effect of dimethoxy group on the phenyl ring.

Effect of fluoro group: Presence of fluoro group at the *meta* position on the phenyl ring (**3i**) make a molecule biologically inactive in nature. While fluoro group substitution at the *para* position on the phenyl ring (**3h**) gives the potent anticancer molecule, inhibiting the growth of breast cancer cell lines, MCF7 and BT-474, *in-vitro.*

Effect of nitro group: The substitution of the Nitro group at the *para* position on the phenyl ring (**3j**) shows promising anticancer activity. It may be due to the electron withdrawing nature of nitro group.

Effect of thiophen-2-yl group: The substitution of thiophen-2-yl group at 2-position on imidazole moiety (**3l**), shows inactive in nature. It may be due to the small size of five member sulfur containing ring.

4. Conclusions

In conclusion, the compounds were synthesized by microwave-irradiations as well as conventional methods with reduced reaction times and increased yields of the products. Among the synthesized compounds, **3h** and **3j** shows prominent anticancer activity against both breast cancer cell lines, MCF-7 and BT-474. While the compounds **3b** and **3k** are specifically active towards bacterium *E. coli* and *B. subtilis*, respectively; more potent than standard drugs used. It should be noticed that some compounds tested exhibited better activity than commercial antimicrobial agents used as reference drugs while there were less active than ciprofloxacin and ampicillin. The some of the compounds showed the worst activity against *S. aureus* followed by *S. typhimurium, P. chrysogenum* and *F. oxysporum*.

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