Aluminium chloride catalyzed one-pot synthesis of 2-aryl substituted benzimidazoles and their antibacterial activity

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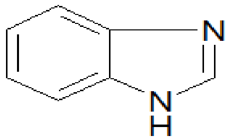
Abstract

2-aryl substituted benzimidazole derivatives were synthesized by using various aromatic aldehydes contains electron releasing as well as electron withdrawing groups, o-phenylenediamine and aluminium chloride as a catalyst via one-pot reaction by use of water as ecological solvent. This route provides an ecological, uncomplicated develops and give compounds in high yield. The synthesized 2-aryl substituted benzimidazole derivatives were characterized by physical (molecular weight, molecular formula, melting point, recrystallization, R_f value) and spectral data (IR and $^{(1)}$H-NMR). All the synthesized compounds were evaluated for their in-vitro antibacterial activity against few gram-positive and few gram-negative microorganisms.

Keywords: *o*-Phenylenediamine, Aluminium chloride, Aromatic aldehydes, 2-Aryl substituted benzimidazole derivatives, Antibacterial activity.

Introduction

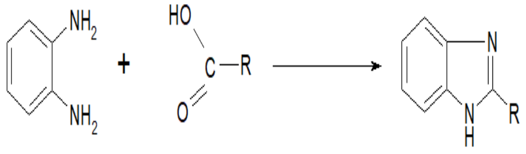
Benzimidazole is an aromatic heterocyclic organic bicyclic compound in which benzene and imidazole fused together. The majority eminent benzimidazole compounds in the natural world is *N*-ribosyl dimethyl benzimidazole and it serves as ligand designed for cobalt in vitamin B_{12}.[(1,2)]



Benzimidazole

Benzimidazole is a colorless to slightly buff colored solid having melting point 172ºC which is soluble in water slightly, freely soluble in ethanol. Benzimidazoles are bicyclic compounds having benzene ring fused with imidazole (contains two nitrogen atoms at 1st and 3rd position). Benzimidazole and their derivatives are employed in various organic compounds synthesis and fungicides which are having ability to inhibit certain microorganisms actions. Various benzimidazole class fungicides comprise debacarb, carbendazim, fuberidazole, chlorfenazole, furophanate, cypendazole, rabenzazole, thiabendazole. Some drugs for example proton pump inhibitors and anthelmintic agents contains benzimidazole nucleus in their structure.

For benzimidazoles, the usual synthesis is the cyclo-condensation of *o*-phenylenediamine/ substituted *o*-phenylenediamines by means of carboxylic acids or their derivatives. At 100°C *o*-phenylendiamine undergo cyclo-condensation with formic acid to give up more than 80% benzimidazole. Carboxylic acids react more slowly with *N*-monosubstituted *o*-phenylenediamines, require addition of HCl or H_3PO_4. Mixture of trifluoro methane sulfonic acid anhydride and triphenyl phosphane oxide in DCM (dichloromethane) is extremely capable dehydrating agent.[(3)]



o-phenylenediamine carboxylic acids 2-substituted benzimidazoles

In 1990, a variety of benzimidazole analogs were developed with replacement of propylene, fluorine, tetrahydro quinoline and cyclized compounds with improved bioavailability, stability and significant physiological/ biological activity.[(4,5)] In 1991, wide range of benzimidazole derivatives were synthesized *via* derivatization at -NH- of benzimidazole with the use of electron donating groups and exchange with extended chain of propyl, thio, acetamido, tetra methyl piperidine on pyridine, thiazolo amino results compounds with good anti-ulcer activity.[(6,7)] Now-a-days contagious microbial diseases involved in causing troubles world-wide, for the reason that they are resistance to wide range of number of anti-microbial agents (vancomycin, β-lactam antibiotics, quinolones, and macrolides). A series of clinically important genus of microbes has develop into a significant health problems internationally.[(8)] Benzimidazoles structural resemblance to purines, anti-bacterial capability is give explanation by their competition by means of purines resulting the inhibition of synthesis of nucleic acids and proteins in case of bacteria.[9,10]

Benzimidazoles spectral properties[11]

1. **Infra red spectrometry properties:** Benzimidazoles gives absorption spectrum nearly at 2850Å designate the existence of aryl moiety, another absorption spectrum nearly at 3107Å designate the presence of -NH- stretch and 1690Å designate the presence of -C-N stretching.
2. **Mass spectrometry properties:** The simple benzimidazoles fragmentation path is similar to the imidazoles fragmentation. Benzimidazoles mass spectrum specifies the sequential loss of two hydrogen cyanide molecules from the molecular ion and the primary of which is non-specific was confirmed by deuterium labeling procedures. Important characteristic aspect in case of fragmentation of 2-n-propyl benzimidazoles is the removal of ethylene from molecular ion. 2-acyl as well as 2-benzoyl benzimidazoles are characterized by loss of CO (carbon monoxide) from the molecular ion.
3. **^{1}H-NMR spectrometry properties:** Important characteristic of this task is with the intention of the protonation factors resultant from simple heterocyclics having five and six membered rings can be utilized to predicting the chemical shift values resulting from protonation of nitrogen and deprotonation in case of more complex structures. Chemical shift values (δ) 7 to 9 shows multiplet due to the presence of aryl ring in benzimidazole.
4. **[13]C-NMR spectrometry properties:** ^{13}C- NMR spectra produce dissimilar peaks of various carbon atoms in the range of δ 0 to 200 when compared with the standard TMS. Especially in case of benzimidazole derivatives it is in the range of δ 115 to 145. Doublet and triplet peaks indicates that there is overlapping. Presence of proton less carbons gives low intensity peaks.

Recent research studies on various benzimidazole derivatives states, they were possess various biological activities like anti-parasitic activity[12], anti-inflammatory activity[13,14], anti-convulsant activity[15], anti-hypertensive activity[16],anti-bacterial and anti-fungal activity[17], anti-viral activity[18], anti-helmenthic activity[19], anti-leishmanial activity[20], anti-diabetic activity[21].

Materials and Instruments

All the chemicals (reagents & solvents) were obtained from commercial suppliers (Merck grade) and they were used further without purification. Melting points were determined by using electrical melting point apparatus and those are uncorrected. Progress of the reaction was monitored by using commercially available pre-coated TLC plates (E. Merck). By using KBr pressed pellet technique IR spectra were recorded on Bruker analyzer. ^{1}H-NMR spectra were recorded on Bruker-400 MHz spectrometer (chemical shifts in δ, ppm) in DMSO using internal standard TMS.

Synthesis of 2-aryl substituted-1H-benzimidazoles[22]

A mixture of o-phenylenediamine (3 mmol) **1**, substituted aryl aldehydes (3 mmol) **2** and 10 mol% of aluminium chloride in 10 ml of water was stirred for about 30 minutes at room temperature. Reaction completion process was monitored by TLC using stationary phase Silica Gel-G and mobile phase ethyl acetate and hexane (1:5). Cool down the reaction mixture and poured onto cold water/ crushed ice. The separated solid was filtered off, washed with cold water. Purify the crude product by recrystallization using 95% ethanol. Scheme of synthesis was depicted in Fig 1.

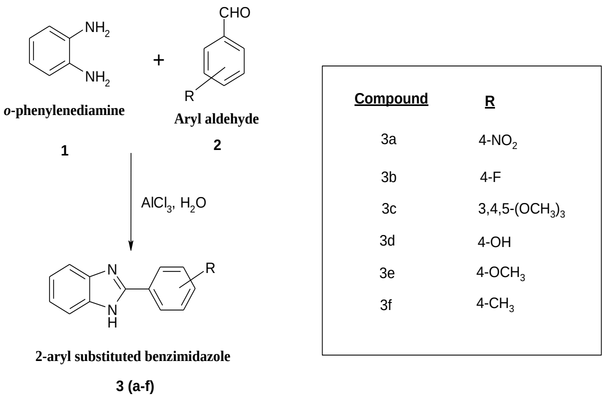


Fig. 1: Scheme of synthesis

Antibacterial activity screening[23,24,25,26]

By two-fold serial dilution method, minimum inhibitory concentrations (MIC) of all the synthesized compounds were tested for their anti-bacterial activity against Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa). All the test compounds and ampicillin as standard was dissolved in dimethyl sulfoxide (DMSO) in a concentration of 1280 μg/mL. Further dilutions of the test compounds and standard were prepared by using DMSO only. All the compounds and standard were tested at various concentrations such as 640, 320, 160, 80, 40, 20 μg/mL and DMSO as a control. To each tube containing sterilized nutrient broth medium (5 ml) drug solution was added. MIC tests carried out in Nutrient broth medium, with an inoculums of (1-2) ×10^{6} Colony Forming Unit/mL (CFU/ml) bacterial strains. The test compounds and standard of Nutrient broth serial tube dilutions inoculated with each bacterial strain were incubated at 37±2ºC for 18-24 h.

The MIC of each test compound was recorded as the lowest concentration in the tubes with no growth (i.e. no turbidity) of inoculated bacterial strains. Nutrient broth medium containing DMSO inoculated microorganisms were used as negative control and nutrient broth medium containing ampicllin inoculated microorganisms were used as positive control. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and MICs were reported.

Results and Discussion

2-aryl substituted-1*H*-benzimidazole analogs were synthesized by one-pot synthesis by means of aluminium chloride as catalyst. Using appropriate synthetic procedure mentioned as above, the benzimidazole derivatives were synthesized i.e. the reactants o-phenylenediamine, aryl aldehyde, catalyst aluminium chloride in aqueous media were taken in a well cleaned round bottom flask and stirred for about 30 min at room temperature. The reaction progress was observed by TLC. At the end of the reaction completion, the reaction mixture was poured into the cold water or onto crushed ice and then recrystallized using ethanol. All the mentioned synthesized compounds were listed in the Table 1. Synthesized compounds physical characterization was carried out and their molecular formula, molecular weight, melting point, percentage yield, R_f values were represented in the Table 2 and their spectral data i.e. IR and ^{1}H-NMR of the synthesized compounds were illustrated in Table 3.

All the above mentioned synthesized compounds were tested for their in-vitro anti-bacterial activity against the both gram-positive and gram-negative bacterial organisms. The MIC values were determined by using serial dilution technique in nutrient broth medium on the basis of presence or absence of turbidity. Ampicillin was used as reference standard compound. Antibacterial activity results (MIC µg/ml) of synthesized test compounds and ampicillin (reference standard) were represented in Table 4. The comparative study of anti-microbial activity screening of the synthesized compounds was given in Fig. 2. All the tested compounds showed MIC values between 40-320 µg/ml. Antibacterial screening of the tested compounds states that some compounds exhibits moderate to good activity. Compound 3b shown good activity against B. subtilis at 40 µg/ml while compound 3b, 3c, 3e shown good activity against S. aureus at 40 µg/ml. Compound 3b, 3d shown moderate activity against E. coli and P. aeruginosa at 80 µg/ml.

Table 1: List of all the 2-aryl substituted-1*H*-benzimidazoles synthesized

Compound	Chemical name of synthesized substituted-1H-benzimidazoles
3a	2-(4'-nitrophenyl)-1*H*-benzimidazole
3b	2-(4'-fluorophenyl)-1*H*-benzimidazole
3c	2-(3',4',5'-trimethoxyphenyl)-1*H*-benzimidazole
3d	2-(4'-hydroxyphenyl)-1*H*-benzimidazole
3e	2-(4'-methoxyphenyl)-1*H*-benzimidazole
3f	2-(4'-methylphenyl)-1*H*-benzimidazole

Table 2: Synthesized compounds physical characterization data

Compound	Molecular formula	Molecular weight (gm)	Melting point (°C)	% of yield	R_f values
3a	$C_{13}H_9N_3O_2$	223	304	69.11	0.64
3b	$C_{13}H_9N_2F$	231	240	86.35	0.42
3c	$C_{16}H_{16}N_2O_3$	284	220	65.62	0.63
3d	$C_{13}H_{10}N_2O$	210	198	67.54	0.52
3e	$C_{14}H_{12}N_2O$	225	228	76.55	0.71
3f	$C_{14}H_{12}N_2$	208	258	77.42	0.58

Table 3: Spectral data of the synthesized compounds

Compound	Spectral data (IR & ^{1}H-NMR)
3a	**IR (KBr, cm^{-1}):** 1667.35 (C=N Str.), 3325.52 (-NH- Str.), 3023.56 (=CH Str.), 1533.26 & 1363.89 (-NO_2 Str.), **^{1}H-NMR (400 MHz, DMSO-*d*$_6$)**: δ 12.875 (s, 1H, -NH-), 7.226-7.248 (t, 2H, imidazole), 7.607-7.629 (d, 2H, imidazole), 8.056-8.088 (d, 2H, phenyl), 8.327-8.352 (d, 2H, phenyl).
3b	**IR (KBr, cm^{-1}):** 1672.23 (C=N Str.), 3354.26 (-NH- Str.), 3012.45 (=CH Str.), 1123.78 (C-F Str.), **^{1}H-NMR (400 MHz, DMSO-*d*$_6$)**: δ 12.953 (s, 1H, -NH-), 7.235-7.254 (t, 2H, imidazole), 7.592-7.614 (d, 2H, imidazole), 7.872-7.895 (d, 2H, phenyl), 8.135-8.159 (d, 2H, phenyl).
3c	**IR (KBr, cm^{-1}):** 1662.55 (C=N Str.), 3348.46 (-NH- Str.), 3021.63 (=CH Str.), 1085.52, 1104.25, 1121.85 (ether C-O Str.), 2933.55 (C-H Str.), **^{1}H-NMR (400 MHz, DMSO-*d*$_6$)**: δ 3.782 (s, 9H, 3,4,5-trimethoxy), 12.012

	(s, 1H, -NH-), 6.932 (s, 2H, phenyl), 7.237-7.256 (t, 2H, imidazole), 7.781-7.803 (d, 2H, imidazole).
3d	**IR (KBr, cm^{-1}):** 1668.75 (C=N Str.), 3339.64 (-NH- Str.), 3023.94 (=CH Str.), 3325.65 (phenolic OH Str.), **^{1}H-NMR (400 MHz, DMSO-*d*$_6$)**: δ 12.899 (s, 1H, -NH-), 5.423(s, 1H, OH), 7.331-7.357 (t, 2H, imidazole), 7.653-7.672 (d, 2H, imidazole), 6.985-7.005 (d, 2H, phenyl), 8.028-8.052 (d, 2H, phenyl).
3e	**IR (KBr, cm^{-1}):** 1666.23 (C=N Str.), 3329.58 (-NH- Str.), 3019.16 (=CH Str.), 1114.52 (ether C-O Str.), 2954.32 (C-H Str.), **^{1}H-NMR (400 MHz, DMSO-*d*$_6$)**: δ 3.867 (s, 3H, OCH_3), 12.022 (s, 1H, -NH-), 7.258-7.282 (t, 2H, imidazole), 7.645-7.670 (d, 2H, imidazole), 7.052-7.071 (d, 2H, phenyl), 7.995-8.017 (d, 2H, phenyl).
3f	**IR (KBr, cm^{-1}):** 1664.48 (C=N Str.), 3372.44 (-NH- Str.), 3009.19 (=CH Str.), 2965.62 (C-H Str.), **^{1}H-NMR (400 MHz, DMSO-*d*$_6$)**: δ 2.456 (s, 3H, CH_3), 12.013 (s, 1H, -NH-), 7.203-7.225 (t, 2H, imidazole), 7.775-7.794 (d, 2H, imidazole), 7.491-7.512 (d, 2H, phenyl), 8.211-8.237 (d, 2H, phenyl).

Table 4: Antibacterial activity of 2-aryl substituted-1*H*-benzimidazoles

Compounds & Standard	**MIC values of tested compounds (µg/ml) against**			
	Gram-positive bacteria		**Gram-negative bacteria**	
	B. subtilis MTCC 1134	*S. aureus* MTCC 1144	*E. coli* MTCC 1089	*P. aeruginosa* MTCC 424
3a	320	80	160	160
3b	40	40	80	80
3c	80	40	320	160
3d	320	160	80	80
3e	320	40	160	320
3f	160	320	160	160
Ampicillin	20	20	20	20

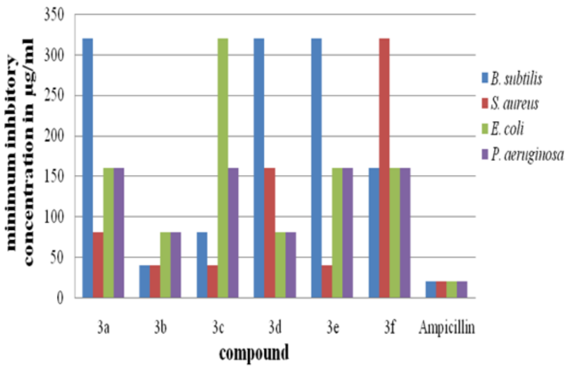


Fig. 1: Comparative antimicrobial activity of the synthesized compounds

Conclusion

In the present work different aryl aldehydes were used to prepare 2-aryl substituted-1*H*-benzimidazole derivatives by cyclization with *o*-phenylenediamine at room temperature in presence of aluminium chloride as catalyst and water as solvent gives good yields. An eco-friendly facile method under mild conditions has been developed for the synthesis of the titled compounds. Among the titled synthesized compounds 2-(4'-fluorophenyl)-1*H*-benzimidazole (3b) shows high % of yield and those were characterized physically to know the molecular weight, molecular formula, melting point, recrystallization, R_f value and spectrally by observing IR and ^{1}H-NMR spectra. Antibacterial screening of the tested compounds revealed that compound 3b consists of 4-fluorophenyl moiety at 2nd position of the benzimidazole shown good activity against gram-positive (*B. subtilis* and *S. aureus*) and gram-negative (E. coli and P. aeruginosa) bacteria at 40 µg/ml, 80 µg/ml respectively. All the remaining compounds showed moderate activity against bacteria at different concentrations, while compound 3b, 3c, 3e shown good activity against *S. aureus* at 40 µg/ml. Compound 3b, 3d shown moderate activity against *E. coli* and *P. aeruginosa* at 80 µg/ml. The MIC values were determined by using serial dilution technique in nutrient broth medium using Ampicillin as a standard drug. All the mentioned synthesized compounds were recognized as less active than the reference standard drug ampicillin.

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