Synthesis and Antibacterial activity of 2-aminobenzimidazole derivatives.

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Abstract: *The reaction of o-Phenylenediamine with Anthranilic acid yield compound 2-(1H-Benzimidazole-2-yl)aniline(1). Compound (1) was condensed with hydrazine hydrate in presence of ethylene glycol at 5^0 C to get compound 2-(2-hydrazinylphenyl)-1H-benzimidazole. Which was then reacted with corresponding aromatic aldehydes to get 2-{2-[(2E)-2-benzylidenehydrazinyl]phenyl}-1H-benzimidazole derivatives. The compounds were synthesized in good yield and their structures were confirmed by IR, NMR and mass spectral data. Synthesized compounds were tested for antibacterial activities.*

Keywords: *2 Aminobenzimidazole, o-Phenylenediamine, Anthranilic acid and biological activity.*

I.   Introduction

Benzimidazole nucleus, which is a useful structure for further molecular exploration and for the development of new pharmaceutical compounds, has been studied extensively. Synthesis of benzimidazoles has received much attention owing to the varied biological activity exhibited by a number of its derivatives[1-5]. The class of these molecules proved to be very important as they possess pharmaceutical properties including antibacterial against different strains of Gram-positive and Gram negative bacteria[6,7], antifungal[8], herbicidal[9], analgesic[10], antioxidant[11,12], antiallergic[13,14], antitumoral agents[15], antiparasitic[16] and anthelmintic[17] etc. Various benzimidazole derivatives have been found to posses bioactivity as phosphodiesterase IV inhibitor[18], antagonist[19] of angiotensin II, neuropeptide Y receptors[20], H3-receptor antagonist[21] and neuropeptide Y5-receptor antagonist[22]. Recently, benzimidazole derivatives have attracted particular interest due to their anticancer activity and may act as in vitro anti-HIV agents[23-25]. Owing to the great biological and synthetic importance of this heterocyclic core, synthesis of benzimidazole derivatives has long been an area of intense development, and still constitutes an active domain from academic and industrial points of view[26].

II.   Materials And Methods

All the chemicals used were of synthetic grade procured from various chemical units like Loba Chemicals, SRL, Chem.Lab, Mumbai. Melting points of all the synthesized compounds were determined in open capillary tubes and the values were uncorrected. The IR spectra were recorded on FT-IR 8101 (Shimadzu) spectrometer by KBr pellets technique. ^{1}H-NMR spectra were recorded on 300 MHz spectrometer using DMSO-d[6] as solvent and TMS as internal standard. The purity of the compounds was checked by TLC on pre-coated silica gel plates by using hexane: ethyl acetate as a mobile phase and visualized in iodine vapour.

III. Experimental

3.1 Preparation of 2-(1H-Benzimidazole-2-yl)aniline (Compound 1)

Heated together a mixture of o-phenylenediamine (5 g, 0.046 mole), 50 mL of 4N HCl, anthranilic acid (6.3g, 0.046 mole) under reflux for 12 hrs. Cooled reaction mixture was made distinctly basic by the gradual addition of the concentrated ammonia solution; the precipitated product was collected and recrystallized from ethanol.

3.2 Preparation of 2-(2-hydrazinylphenyl)-1H-benzimidazole (Compound 2)

Concentrated HCl (1ml) was added drop wise with stirring to hydrazine hydrate (1ml, 0.00478mol) at $5-10^0$c followed by ethylene glycol (1ml). To the above solution substituted benzimidazole (0.004mol) was added in portion and the resulting mixture was refluxed for 2 hrs, cooled, poured in crushed ice. The solid separated, was filtered, dried and recrystallized from methanol.

3.3 Preparation of 2-{2-[(2*E*)-2-benzylidenehydrazinyl] phenyl}-1*H*-benzimidazole (Compound 3)
A. Conventional heating method:

In a round bottom flask, Compound 2 (1 mol) and substituted benzaldehyde were taken in ethanol. The reaction mixture was reflux for about 8 hrs till the completion of the reaction. Progress of the reaction was checked with TLC (Hexane: Ethyl acetate – 4:1) Then it was cooled with ice cold water. It was filtered and washed with cold water and dried, crude product was recrystallized from ethanol.

B. Microwave irradiation method:

A mixture of Compound 2(1mol), substituted benzaldehyde (0.004mol) and few drops of DMF were added in a hard glass tube and irradiated in microwave oven at appropriate power and time. Completion of the reaction was monitored by TLC, mixture was cooled and poured with ice cold water. And the resulting Solid formed was filtered, dried and recrystallized from ethanol.

2-{2-[(2*E*)-2-benzylidenehydrazinyl]phenyl}-1*H*-benzimidazole(**3a**): IR (KBr) cm^{-1}: 3417 (-NH); 3163 (Ar-CH.);2947 (CH); 1620(Ar-C=C), 748,(Ar-CH), ^{1}H-NMR (DMSO d^6) δ: 9.0 (1H, NH), 4.5(1H,s,OH),10.2(1H,s,NH),10.0 (1H,s,NH),8.6(1H, s,CH=),6.8-8.1 (12H)m, MS:- m/z = 312(m+)
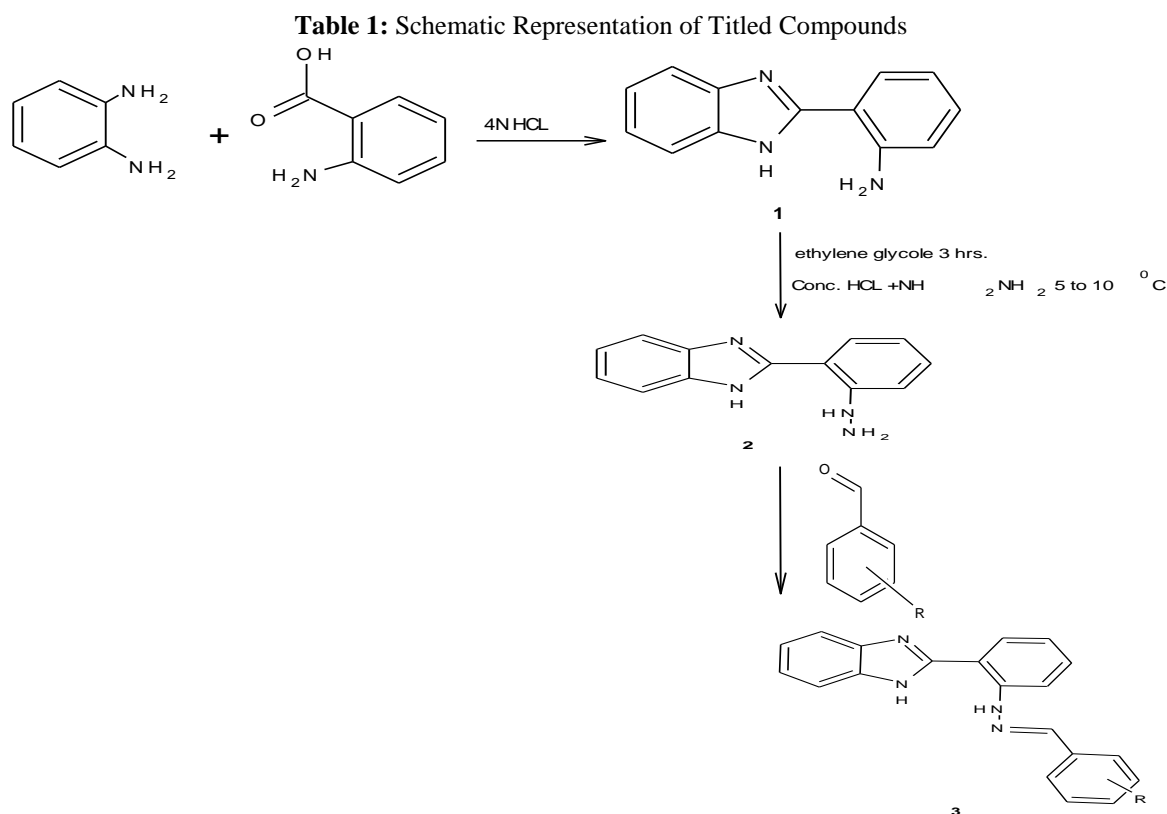
Table 1: Schematic Representation of Titled Compounds

Where R=H, -Cl. CH$_3$, -Cl, -NO$_2$, -OC$_2$H$_5$ etc.

Table 2: Comparative table Conventional methods and Microwave Irradiation Methods.

Entry	R	Conventional heating		Microwave heating			mp. °C
		Time in Hours	% Yields*	Microwave power in Watt	Time in min.	% yield*	
3a	H	8	55	300	3	88	92
3b	4-N,N-di- CH$_3$	8	61	300	2.5	91	190
3c	2-OH	8	57	300	3	87	195
3d	4-OH	8	63	300	3.5	85	230
3e	4-OCH$_3$	8	61	300	3.5	86	165
3f	furfuraldehyde	8	52	300	4.5	79	139
3g	1-Naphthaldehyde	8	54	300	5	76	160
3h	4-OH,3-OCH$_3$	8	58	300	4.5	86	235
3i	3- NO$_2$	8	52	300	6	75	97
3j	3-Br	8	61	300	3	86	135

· *Yield refer to purified compounds

Antibacterial Bioassay

0.4 % of the MIC (minimum inhibitory concentration) of all the final products were prepare in dimethylformamide solvent and tested against one Gram +ve (Escherichia Coli) and one gram –ve bacteria (Staphylococcus Aureus). The composition of nutrient agar medium was bactotryptone (4g), Broth (3.9 g) less than 2%, NaCl (2.9 g) in 100 ml of water (2.9%). After 18h the exponentially growing culture of the 2 bacteria in nutrient broth at 37^0C were diluted in sterile broth. From each of these diluted culture, 1ml was added to 100 ml sterilized and cooled nutrient agar media to give a final bacterial culture. The plates were set at room temperature and later dried at 37^0C for 20h. Paper discs (6mm, punched from whatmann no. 41 paper) used for the assays. Discs were socked in DMF and placed on the inoculated agar media at regular intervals of 6-7 cm, care was taken to ensure that excess solution was not on the discs. All the samples were taken in triplicates. The plates were incubated at 37^0C in an inverted fusion. Activity was determined by zone showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control.

IV. Results

The structure of synthesized compounds was confirmed by IR, ^{1}H NMR, and GC-MS analysis. Compounds (3a-j) were screened against two pathogenic bacteria. One Gram negative strains viz., *Escherichia Coli* and one Gram positive stains viz., *Staphylococcus Aureus* following agar well diffusion procedure as per the reference. The antibacterial activity of the synthesized benzimidazole 3a-j was corrected with the zone of inhibition of erythromycin as a standard control. (Table 2). The bacterial test result for the newly synthesized benzimidazole analogues revealed that most of the compounds exhibited moderated to good activity against Gram +ve (*Staphylococcus Aureus*) and Gram –ve bacteria (*Escherichia Coli*). For *Staphylococcus Aureus* compounds 3a and 3g exhibited maximum activity while the other compounds displayed moderate activity. And in case of *Escherichia Coli,* compounds 3e and 3g exhibited good to excellent activity while the reaming compounds displayed moderate and less activity. As all compounds showed antibacterial activity against the bacteria tested. It indicates that this basic moiety can be a potential scaffold for antibacterial drugs. It may be suggested that the amino benzimidazole derivative with a suitable R group may lead to a good antibacterial agent for all the *Escherichia Coli* and *Staphylococcus Aureus* bacterial strains. Thus further lead optimization is required to get wide spectrum of activity.

Table 2: Result of Antibacterial Bioassay of compounds

Compounds no.	Zone of Inhibition (mm)		
		Gram -ve	Gram +ve
	R	**E.coli**	**S. aureus**
3a	H	14	12
3b	4-N,N-di CH$_3$	11	08
3c	2-OH	13	11
3d	4-OH	14	12
3e	4-OCH$_3$	16	10
3f	furfuraldehyde	13	12
3g	1-Naphthaldehyde	18	14
3h	vanilin	11	10
3i	3- NO$_2$	12	09
3j	3-Br	14	12
Standard	**Erythromycin**	24	22

V. Conclusion

Substituted derivatives of 2-{2-[(2*E*)-2-benzylidenehydrazinyl]phenyl}-1*H*-benzimidazole (3a-j) were prepare from commercially available 2- amino benzimidazole and tested for Gram positive and Gram Negative bacterial cultures. All the compounds were found to exhibit good to moderate antibacterial activity against different strains of bacteria. It was observed that among all the compounds tested. Compounds 3i showed good activity (having nitro substituent) against all the tested bacterial strains and remaining compounds such as 3a, 3b,3c,3d,3f displayed moderated activity.

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