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ANTIBACTERIAL EVALUATION OF SOME BENZIMIDAZOLE AZOMETHINE DERIVATIVES SYNTHESIZED *VIA* GREENER APPROACH

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ABSTRACT:
The interaction of substituted Benzimidazole (**A1, A2**) and aromatic aldehyde (**B1, B2**) *via* a greener approach resulted in the synthesis of azomethine derivatives (**C1 – C4**). The compounds **A1** and **A2** were synthesized using phenylenediamine and respective carboxylic acids using aqueous media. The resultant compounds were characterized by elemental analysis, ^{1}H-NMR and IR spectral studies.
The *in-vitro* Antibacterial activities of the final compounds have proven that they are active against the bacterial strains (*B. subtilis, S. aureus, P. aeruginosa* and *E. coli*) incorporating microdilution method.

KEYWORDS: Azomethine, Green synthesis, Benzimidazoles, Antibacterial activities.

INTRODUCTION:

Azomethines have been widely studied as they can be easily synthesized by the condensation of amines and carbonyl compounds; where the >C=O (carbonyl group) gets replaced by the >C=N (azomethine group).[i-iii] Azomethine derivatives of Benzimidazoles are significant in medicinal chemistry owing to the Benzimidazole ring bio-activities *viz.*, antimicrobial, antitumor, antihypertensive, antimalarial, antiprotozoal, antiviral, anti-inflammatory, etc. (Figure 1).[iv-xi] It has been proven that the N-atom of the azomethine moiety is associated with the formation of H-bond along with active centers and inhibits normal cell processes.[xii, xiii]
Keeping in view the variety and numerous applications of azomethine entities and Benzimidazoles in different fields of chemistry, there has always been modifications and developments leading to efficient, economical and greener methods for their synthesis.[xiv-xxi]
In addition to aforementioned findings; this research paper involves outcomes on the synthesis, characterization and *in-vitro* antibacterial evaluation of azomethine analogues obtained from substituted Benzimidazoles and aromatic aldehydes. The synthesized analogues have proven that they are active against the bacterial strains for which they were investigated.

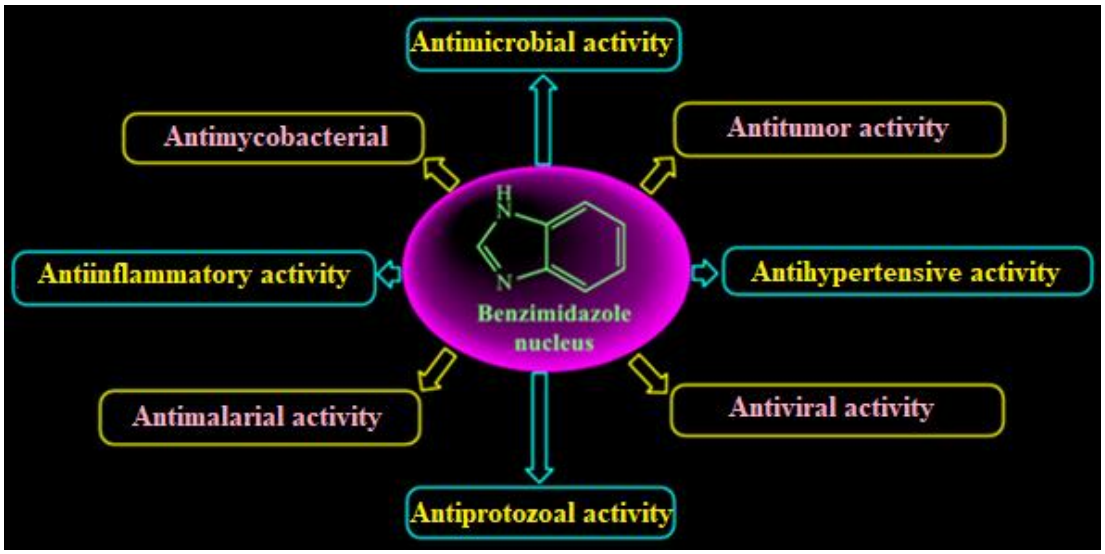


Figure 1. Biological significance of Benzimidazole Derivatives

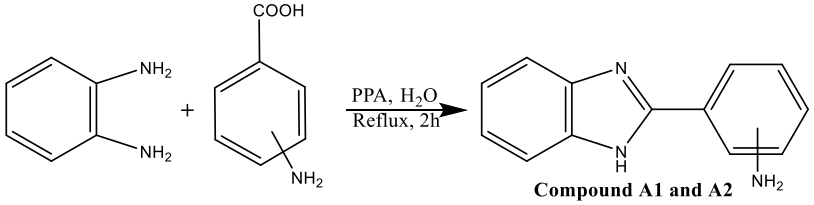
EXPERIMENTAL:

All the commercial chemicals and reagents (procured from Sigma Aldrich/Merck) were used as such without further purification. Carbon, Nitrogen and Hydrogen were analyzed on a Perkin-Elmer C, H, N and S II series 2400 analyzer. IR spectra were recorded using KBr pellet method on a Spectrum Version: 10.4.00 - Perkin Elmer FTIR spectrophotometer in the range 4000-400cm^{-1}. ^{1}H-NMR spectra were recorded for the synthesized compounds using TMS as the internal reference on Bruker Ascend 300 MHz system in d_6-DMSO. Melting points were determined using an electric melting point apparatus and were uncorrected.

Green SynthesisofSubstituted Benzimidazoles(Scheme 1)

A mixture of m-aminobenzoic acid (1mmol) and o-phenylenediamine (1mmol) in PPA (polyphosphoric acid) was refluxed in water for about 2 hours. The obtained dark precipitate was filtered under vacuum, washed twice with water and dried in electric oven for about 3-4 hours to obtain compound **A1** as powdered solid (m.p.: 257-259˚C, Yield: 91%).

A similar procedure with p-aminobenzoic acid resulted in synthesis of compound **A2** (m.p.: 241-243˚C, Yield: 82%).

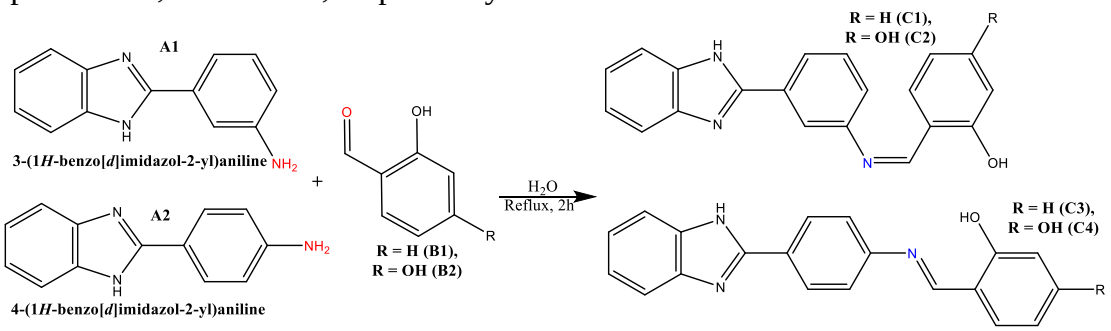


Scheme 1. Green Synthesis of Substituted Benzimidazole

Green Synthesis of Azomethine Derivatives (Scheme 2)

A mixture of Benzimidazole (**A1**, 1mmol) and salicyladehyde (**B1**, 1mmol) in water was refluxed for about 2 hrs. The obtained precipitate was filtered under vacuum, washed twice with water and dried in electric oven for about 4 hours to obtain compound **C1** (m.p.: 232-234˚C, Yield: 87%).

A similar procedure with reactant pairs **A1-B2, A2-B1** and **A2-B2** resulted in synthesis of compounds **C2**, **C3** and **C4**; respectively.



Scheme 2. Green Synthesis of Azomethine Derivatives

RESULT AND DISCUSSION:

The synthesis of azomethine derivatives was carried out *via* greener approach in aqueous media in a two-step process. In the very first step, Benzimidazoles (**A1** and **A2**) were prepared through a condensation pathway (Scheme1). Subsequently, azomethine entities were prepared using Benzaldehyde derivatives (**B1** and **B2**) (Scheme 2). All the resultant final compounds (**C1-C4**) were obtained as yellow powdered solids. They were found to be thermally stable and were obtained in 79-93% yield. Physical and analytical observations are listed in Table 1.

Table 1

Physical and Analytical data for the synthesized compounds

S. No.	Compound	Molecular Formula	M.P. (°C)	Yield (%)	Elemental Analysis % found (% calcd.)		
					C	H	N
1	Benzimidazole – **A1**	$C_{13}H_{11}N_3$	257-259	91	74.56 (74.62)	5.27 (5.30)	20.02 (20.08)
2	Compound - **C1 (R = H)**	$C_{20}H_{15}N_3O$	232-234	87	76.94 (76.66)	4.89 (4.83)	13.33 (13.41)
3	Compound – **C2 (R = OH)**	$C_{20}H_{15}N_3O_2$	282-284	93	73.15 (72.94)	4.55 (4.59)	12.52 (12.76)
4	Benzimidazole - **A2**	$C_{13}H_{11}N_3$	241-243	82	74.66 (74.62)	5.25 (5.30)	20.09 (20.08)
5	Compound – **C3 (R = H)**	$C_{20}H_{15}N_3O$	244-246	86	77.06 (76.66)	4.93 (4.83)	13.39 (13.41)
6	Compound – **C4 (R = OH)**	$C_{20}H_{15}N_3O_2$	262-264	79	72.76 (72.94)	4.64 (4.59)	12.61 (12.76)

IR Spectra (Table 2)

IR Spectra of the synthesized Benzimidazoles and their azomethine derivatives were observed in the definite region of 4000-400 cm^{-1}. These spectra were analyzed depending on some key peaks as recorded. The formation of azomethine was verified by the absence of peaks in the range 3450-3250 cm^{-1} attributed to amino group ν(-NH_2) in **A1** and **A2**; and formation of particular band around 1610-1590 cm^{-1} characteristic for imine group in **C1**, **C2**, **C3** and **C4**.[xxii-xxiv]

The signals in the region 1275-1260 $cm^{-1}$and 3065-3045 cm^{-1} in spectra of derivatives **C1-C4** have been accounted for ν(C–O, phenolic) and ν(OH, phenolic), respectively. The peaks

recorded in the spectra around region 1585-1560 cm^{-1} are attributed to ν(N=C) of the Benzimidazole ring present in all compounds.

Table 2
IR spectral readings forthe synthesized compounds

S. No.	Compound	υ(NH_2)	υ(OH)	υ(N=CH)	υ(N=C)	υ(C=C)	υ(C-O)
1	Benzimidazole – **A1**	3445, 3340	---	---	1570	1505	---
2	Compound - **C1**	---	3055	1605	1580	1510	1260
3	Compound – **C2**	---	3065	1610	1585	1500	1260
4	Benzimidazole - **A2**	3405, 3335	---	---	1560	1490	---
5	Compound – **C3**	---	3045	1595	1565	1500	1275
6	Compound – **C4**	---	3060	1600	1570	1510	1275

^{1}H-NMR Spectra (Table 3)
The presence of signals observed in 9.07-8.89 ppm region in the proton spectra of compounds **C1**, **C2**, **C3** and **C4** can be attributed to N=CH group; which clearly implies the formation of azomethine bond.[xxv, xxvi] Additionally, signals observed in the typical ppm region are assigned to aromatic protons in the proton spectra of all the compounds. Signals observed in the low field in compounds **C1-C4** around 13.49-12.93 ppm region have been assigned to the NH, OH protons of Benzimidazole and Salicylidene rings, respectively.

Table 3
^{1}H-NMR spectral readings for the synthesized compounds

S. No.	Compound	^{1}H NMR (300 MHz, Me_2SO–d_6)
1	Benzimidazole – **A1**	δ 12.67 (s, 1H), 7.59 (d, 1H), 7.46 (d, 1H), 7.40 (s, 1H), 7.25 (d, 1H), 7.20–7.08 (m, 3H), 6.65 (d, 1H), 5.27 (s, 2H)
2	Compound - **C1**	δ 12.95 (s, 1H), 12.95 (s, 1H), 9.06 (s, 1H), 8.16 (s, 1H), 8.08 (d, 1H), 7.69 (dd, 1H), 7.61 (m, 4H), 7.42 (dd, 1H), 7.19 (dd, 2H), 7.00 (t, 2H)
3	Compound – **C2**	*δ* 13.42 (s, 1H), 12.93 (s, 1H), 10.29 (s, 1H), 8.89 (s, 1H), 8.09 (t, 1H), 8.03 (dd, 1H), 7.64 (d, 1H), 7.56 (t, 1H), 7.51 (d, 1H), 7.46 (d, 1H), 7.44 (d, 1H), 7.18 (m, 2H), 6.40 (dd, 1H), 6.30 (s, 1H)
4	Benzimidazole - **A2**	*δ* 12.45 (s, 1H), 7.87 (d, 2H), 7.50 (s, 2H), 7.14 (dd, 2H), 6.76–6.51 (m, 2H), 5.62(s, 2H)
5	Compound – **C3**	*δ* 12.97 (s, 1H), 12.96 (s, 1H), 9.07 (s, 1H), 8.26 (d, 2H), 7.68 (m, 1H), 7.60 (d, 3H), 7.43 (dd, 1H), 7.21 (dd, 2H), 6.99 (t, 2H), 6.98 (d, 1H)
6	Compound – **C4**	*δ* 13.49 (s,1H), 12.95 (s,1H), 10.37 (s,1H), 8.96 (s,1H), 8.28 (m,2H), 7.70 (m, 1H), 7.58 (m, 3H), 7.51 (d, 1H), 7.25 (t, 2H), 6.47 (dd, 1H), 6.36 (d, 1H)

Antibacterial Evaluation (Table 4, Figure 2)

The synthesized azomethine derivatives were evaluated for their antibacterial activities against the bacterial strains (*B. subtilis* ATCC 55406, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 9027 and *E. coli* ATCC 8739). For this purpose; Ciprofloxacin was used as the control drug for antibacterial activity.[xxvii, xxviii]

Antibacterial evaluation of compounds **C1-C4** reveals that they exhibit appreciable activities against Gram-positive (*B. subtilis* and *S.aureus*) as well as Gram-negative (*P. aeruginosa* and *E. coli*) bacterial strains. Strains were incubated with the compounds under investigation at 37 ˚C for 48 hours and the assays were performed in triplicate.

Table 4

Antibacterial activityresultsfor the synthesized compounds **C1-C4**

S. N.	Compound	MIC concentrations (µg/ml)			
		Gram-positive bacterial strains		Gram-negative bacterial strains	
		B. subtilis	***S. aureus***	***P. aeruginosa***	***E. coli***
1	**C1**	450	450	1750	1500
2	**C2**	400	400	1500	1000
3	**C3**	250	225	750	500
4	**C4**	750	225	1500	750
5	**Ciprofloxacin**	1	1	10	5

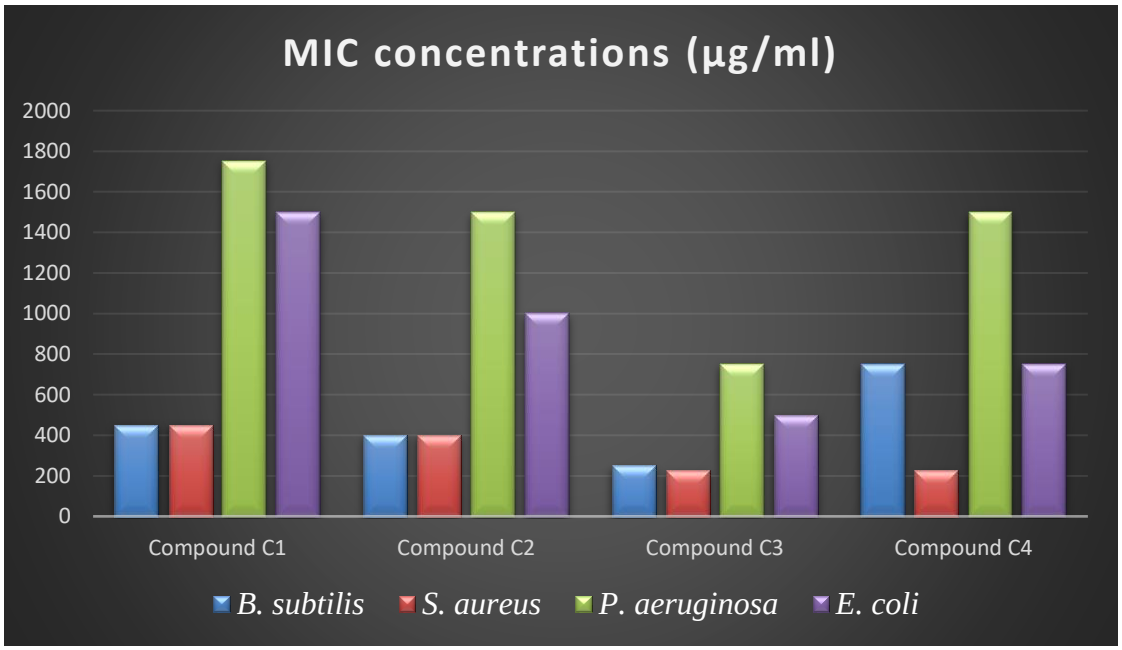


Figure 2. Antimicrobial activities of synthesized compounds C1-C4

CONCLUSION

In the mentioned work here; green synthesis of some azomethine derivatives obtained from substituted Benzimidazole and aromatic aldehydes have been reported. All the synthesized analogues were analyzed based on their elemental analysis, ^{1}H-NMR, IR spectral studies. The antibacterial evaluation of azomethine derivatives demonstrates their significant biological activities against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*.

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