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Exploration of in vitro time point quantitative evaluation of newly synthesized benzimidazole and benzothiazole derivatives as potential antibacterial agents

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ABSTRACT

Present communication deals with the in vitro time point quantitative antibacterial evaluation of newly synthesized 1,2-disubstituted benzimidazoles (**3a–p**) and 2-substituted benzothiazoles (**5a–h**) against Gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, and Gram-negative bacteria *Vibrio cholerae*, *Shigella dysenteriae* and *Escherichia coli*. These compounds were synthesized under mild reaction conditions using Al_2O_3–Fe_2O_3 nanocrystals as heterogeneous catalyst. Bio-evaluation studies revealed that, compounds **3a**, **5a** and **5d** exhibited moderate to good antibacterial activity against all the tested bacterial stains. The compounds **3a**, **3f** and **5a** have shown enhanced inhibitory activity compared with standard antibacterial drug ciprofloxacin against *V. cholerae*, *B. cereus*, and *S. dysenteriae*, respectively. Additionally, the compounds **3a**, **3e**, **3f**, **3h** and **5b** displayed complete bactericidal activity within 24 h, whereas ciprofloxacin took 48 h to kill those bacteria completely.

Infectious as well as highly contagious microbial diseases are increasing with course of time round the world due to the emergence of new multidrug resistant bacteria which are resistant to a number of antimicrobial agents due to the development of mutagenicity.[1] One way to battle with this challenge is the conscious usage of the currently marketed antibiotics and the other is to develop and screen new chemical entities for antimicrobial activities.[2] In view of this, it is imperative to discover new chemotherapeutic agents to prevent the emergence of resistance and ideally shorten the duration of therapy.

Benzimidazoles and benzothiazoles are the important pharmacophore and privileged sub-structures in medicinal chemistry owing to their involvement as a key component for various biological activities.[3–8] The high profile of biological applications displayed by compounds associated with these nuclei have prompted wide studies for their synthesis.[9–20] Extensive biochemical and pharmacological studies have confirmed that benzimidazole and benzothiazole derivatives are effective against various strains of microor ganisms. Due to the structural similarity with purine, antibacterial ability of these compounds manifested their competition with purines resulting in the distinct inhibition of the synthesis of nucleic acids and proteins inside the bacterial cell wall.[21,22] The therapeutic importance of these class of compounds inspired us to develop a series of 1,2-disubstituted benzimidazoles and 2-substituted benzothiazoles in order to study their antibacterial activities. Recently, we explored the use of mesoporous mixed metal oxide nanocrystals as heterogeneous catalysts for the synthesis of novel series of 1,2-disubstituted benzimidazoles and 2-substituted benzothiazoles.[23] In continuation of our studies on bioactive compounds,[24] herein we report the in vitro qualitative as well as time point quantitative antibacterial activity evaluation of the synthesized benzimidazole and benzothiazole derivatives bearing various functional groups of electron-withdrawing and electron-donating properties against the representative genera of Gram-positive and Gram-negative bacteria.

The synthetic pathways leading to 1,2-disubstituted benzimidazoles (**3a–p**) and 2-substituted benzothiazoles (**5a–h**) via one-pot condensation reaction between aromatic and aliphatic aldehydes **2** with 1,2-phenylenediamine **1** and 2-aminothiophenol **4**, respectively, using Al_2O_3–Fe_2O_3 nanocrystals are depicted in Scheme 1. The structures of the synthesized compounds were confirmed by ^1H and ^{13}C NMR, ESI-MS spectral data, and elemental analysis. The ^1H NMR spectra of all substances displayed multiplets in the aromatic region indicating the presence of the benzimidazole and benzothiazole ring. The mass spectra (ESI-MS) of the

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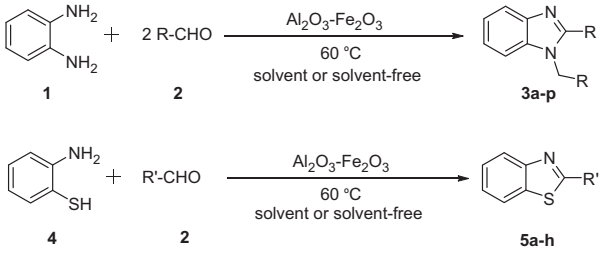

Scheme 1. Synthesis of 1,2-disubstituted benzimidazoles (**3a**–**p**) and 2-substituted benzothiazoles (**5a**–**h**) using Al_2O_3–Fe_2O_3 nanocatalyst.

compounds showed $(M+H)^+$ peaks, in agreement with their molecular weight. Elemental analysis results for C, H and N elements were satisfactory within ±0.4% calculated values of the compounds.

In order to study the catalytic activity, nanocrystalline Al_2O_3–Fe_2O_3 was synthesized by aerogel technique and studied as heterogeneous catalyst for the preparation of a series of 1,2-disubstituted benzimidazoles and 2-substituted benzothiazoles. When the reaction was carried out with 1,2-phenylendiamine and aldehyde in 1:1 molar ratio in presence of Al_2O_3–Fe_2O_3 (5 wt % of 1,2-phenylenediamine), then a mixture of mono and di-substituted product were formed. On the other hand, when 1:2 molar ratio was used then the desired 1,2-disubstituted benzimidazole was formed[25] with excellent selectivity and yields. However, trace amount of monosubstituted product was also formed in few cases, which was separated by column chromatography. To demonstrate the synthetic potential of this protocol the same procedure was extended for one-pot synthesis of benzothiazole[25] class of compounds. An overview of synthetic highlights delineating the synthesis of 1,2-disubstituted benzimidazoles and 2-substituted benzothiazoles is depicted in Tables 1 and 2, respectively.

It is evident from Tables 1 and 2 that, the proposed synthetic procedure works well with mono, di (Table 1, entries 5, 6 and 9 and Table 2, entries 5, 6) and even tri-substituted (Table 2, entry 3) aryl aldehydes. The effect of substitution present on aromatic aldehyde on the reaction rate and the overall yield was also studied. As shown, a variety of benzaldehydes bearing electron-donating (Table 1, entries 2–6 and Table 2, entries 1–7) and electron-withdrawing substituents (Table 1, entries 13, 14 and Table 2, entry 8) were successfully employed to prepare the corresponding benzimidazoles and benzothiazoles derivatives in good to excellent yields (81–89%). Furfural (Table 1, entry 10) and cinnamyl aldehyde (Table 1, entry 11) also afforded the desired products in excellent yields (84–90%). Furthermore, this synthetic approach was extended to aliphatic aldehydes, such as heptanal to get the desired 1,2-disubstituted benzimidazole (Table 1, entry 12) with reasonably good yield (82%).

Table 1
Synthesis of 1,2-disubstituted benzimidazoles (**3a**–**p**) using Al_2O_3–Fe_2O_3 nanocatalyst

Entry	R	Product	Time[a](min)	Yield[b] (%)
1	C_6H_5	**3a**	12	84
2	4-MeC_6H_4	**3b**	27	85
3	4-$(Me)_2NC_6H_4$	**3c**	45	83
4	4-$(Me)_3CC_6H_4$	**3d**	22	81
5	3-EtO-4-HOC_6H_3	**3e**	35	89
6	5-Br-2-HOC_6H_3	**3f**	27	85
7	4-FC_6H_4	**3g**	21	88
8	4-$F_3COC_6H_4$	**3h**	25	84
9	3,4-$Cl_2C_6H_3$	**3i**	14	85
10	Furyl	**3j**	12	90
11	C_6H_5CH=CH	**3k**	37	84
12	CH_3-$(CH_2)_4$-CH_2	**3l**	24	82
13	3-$NO_2C_6H_4$	**3m**	10	87
14	4-$F_3CC_6H_4$	**3n**	34	83
15	4-BrC_6H_4	**3o**	24	81
16	4-HOC_6H_4	**3p**	10	82

[a] All the reactions monitored by TLC.
[b] Isolated yield.

Table 2
Synthesis of 2-substituted benzothiazoles (**5a**–**h**) using Al_2O_3–Fe_2O_3 nanocatalyst

Entry	R′	Product	Time[a] (min)	Yield[b] (%)
1	2-EtC_6H_4	**5a**	22	85
2	4-HOC_6H_4	**5b**	16	84
3	3,4,5-$(MeO)_3C_6H_2$	**5c**	33	83
4	4-$EtOC_6H_4$	**5d**	15	85
5	4-EtO-3-$MeOC_6H_3$	**5e**	24	83
6	5-Br-2-$MeOC_6H_3$	**5f**	28	89
7	4-$(Me)_2HCC_6H_4$	**5g**	17	87
8	4-$NO_2C_6H_4$	**5h**	23	88

[a] All the reactions monitored by TLC.
[b] Isolated yield.

The antibacterial activity (zone of growth inhibition) of the tested 1,2-disubstituted benzimidazoles (**3a**–**p**) and 2-substituted benzothiazoles (**5a**–**h**) in comparison with that of control drugs ciprofloxacin was determined by Kirby–Bauer disc diffusion method on Mueller–Hinton agar according to the guidelines[26] of Clinical Laboratory Standards Institute, 2007, USA (Table 3). Among all the tested compounds, many compounds exhibited moderate to excellent activity. The compound **3n** showed moderate activity (zone of growth inhibition 22 mm) against *Escherichia coli* MTCC 1610 as compared to the standard drug (32 mm). Compounds **3a** exhibited excellent efficacy (33 mm) against tested *Vibrio cholerae* MTCC 3904, while the zone of growth inhibition for compounds **3d** and **3h** (21 and 23 mm) was nearly equal to the control drug ciprofloxacin (24 mm). Likewise, against *Shigella dysenteriae* NICED, compound **5a** displayed highest inhibitory activity profile (35 mm) and compounds **3a**, **3l, 3n** and **5c** showed enhanced growth inhibition (19–23 mm) as compared to the standard drug. Only two compounds **3e** and **3n** demonstrated better activity profile (zone of growth inhibition up to 22–23 mm) against Gram-positive bacteria *Staphylococcus aureus* MTCC 3160. Interestingly, in case of *Bacillus cereus* MTCC 430, large number of compounds (**3a**, **3f**, **3g**, **5a**, **5b**, **5d**, **5f** and **5g**) displayed inhibitory activity (18–33 mm) higher than that of ciprofloxacin (14 mm). Among them compound **3f** exhibited the maximum inhibition (33 mm). It is evident from the above table that, compounds **3a**, **5a** and **5d** exhibited moderate to good antibacterial activity against both Gram-positive as well as Gram-negative bacteria. It is noteworthy to mention that, compound **5c** was found to be selectively active against shigellosis causing bacteria *S. dysenteriae* NICED.

Further, upon close inspection of results depicted in Table 3, it has been inferred that, the Gram-positive bacteria were more susceptible towards the newly synthesized series of compounds as compared to the Gram-negative bacteria. This may be due to the absence of a unique outer membrane in Gram-positive bacteria and hence, the wall of Gram-positive bacteria is permeable to these derivatives. Generally, the Gram-positive bacteria are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier[27] whereas the Gram-negative bacteria possess an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to drug constituents.[28]

The results of the quantitative assay for the antibacterial activity of the selected synthesized compounds against *B. cereus* MTCC 430, *S. aureus* MTCC 3160, *V. cholerae* MTCC 3904, *E. coli* MTCC 1610 and *S. dysenteriae* NICED at different time point are graphically represented in Figure 1 [(i)–(v)]. The time point assay[29]

Table 3
Antibacterial activity of compounds: diameter of zone of growth inhibition (mm) against five different bacterial strains based on Kirby–Bauer method

Compounds	R (R′)	Gram-negative bacteria			Gram-positive bacteria	
		E. coli MTCC 1610	*V. cholerae* MTCC 3904	*S. dysentriae* NICED	*S. aureus* MTCC 3160	*B. cereus* MTCC 430
3a	C_6H_5	19	33	23	10	22
3b	4-MeC_6H_4	–	18	13	12	–
3c	4-$(Me)_2NC_6H_4$	–	17	16	11	–
3d	4-$(Me)_3CC_6H_4$	17	21	13	–	12
3e	3-EtO-4-HOC_6H_3	14	–	–	23	–
3f	5-Br-2-HOC_6H_3	17	–	–	–	33
3g	4-FC_6H_4	–	–	–	10	18
3h	4-$F_3COC_6H_4$	–	23	11	10	–
3i	3,4-$Cl_2C_6H_3$	–	–	11	–	–
3j	Furyl	–	13	12	10	–
3k	$C_6H_5CH{=}CH$	–	11	–	–	14
3l	CH_3-$(CH_2)_4$-CH_2	–	16	21	12	15
3m	3-$NO_2C_6H_4$	–	13	–	10	–
3n	4-$F_3CC_6H_4$	22	13	19	22	–
3o	4-BrC_6H_4	–	16	13	–	15
3p	4-HOC_6H_4	–	–	11	–	–
5a	2-EtC_6H_4	11	16	35	13	21
5b	4-HOC_6H_4	–	18	–	–	19
5c	3,4,5-$(MeO)_3C_6H_2$	–	–	23	–	–
5d	4-$EtOC_6H_4$	12	15	16	16	22
5e	4-EtO-3-$MeOC_6H_3$	–	16	15	12	–
5f	5-Br-2-$MeOC_6H_3$	–	17	–	12	21
5g	4-$(Me)_2HCC_6H_4$	–	–	15	13	20
5h	4-$NO_2C_6H_4$	–	–	13	–	14
Ciprofloxacin[a]	–	32	24	14	15	14

[a] Antibacterial activity of the synthesized compounds was compared with the standard antibacterial drug ciprofloxacin.

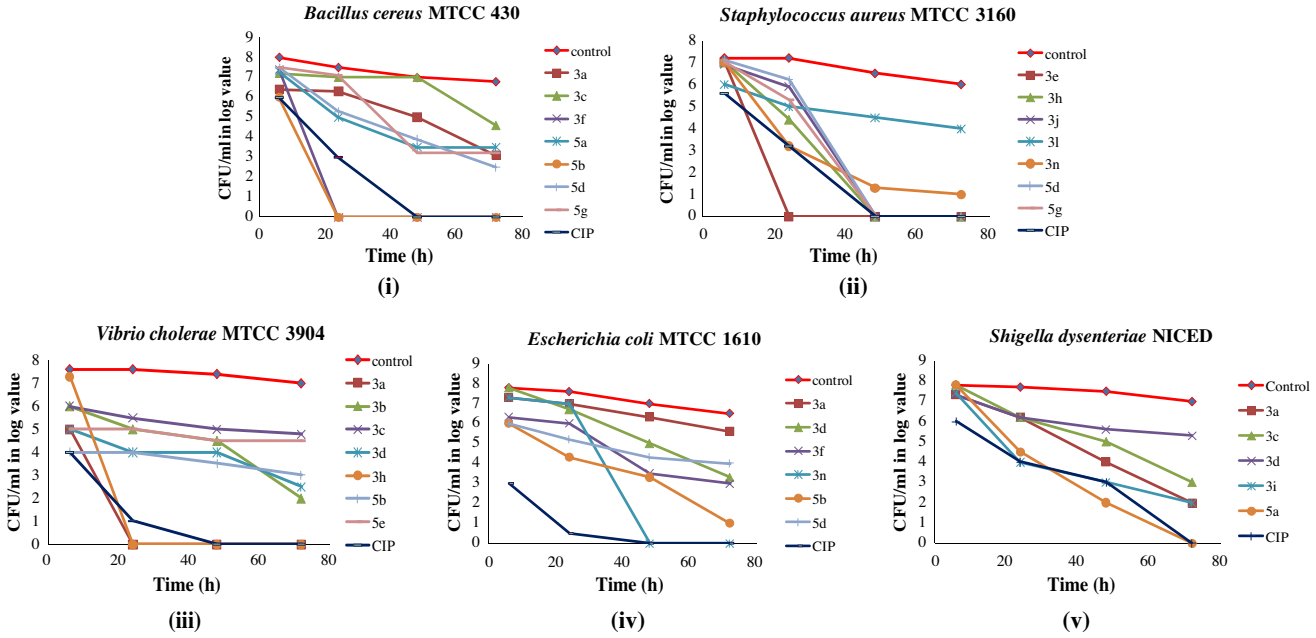

Figure 1. Graphical representation of quantitative antibacterial activity study against (i) *B. cereus* MTCC 430; (ii) *S. aureus* MTCC 3160; (iii) *V. cholerae* MTCC 3904; (iv) *E. coli* MTCC 1610 and (v) *S. dysenteriae* NICED.

results revealed that, the compounds **3f** and **5b** exhibited complete bactericidal effect on *B. cereus* MTCC 430 within 24 h, whereas the compounds **3a**, **5a**, **5d**, **5g** diminished approximately 3–4 log values of the bacterial concentration within 48 h of incubation. In the case of *S. aureus* MTCC 3160, only the compound **3e** completely exterminated the bacteria within 24 h, but compounds **3h**, **3j**, **5d** and **5g** were able to prevent the bacteria completely within 48 h. Against Gram-negative bacteria *V. cholerae* MTCC 3904, the compounds **3a** and **3h** killed the bacteria completely within 24 h, whereas against *E. coli* MTCC 1610, only compound **3n** able to eliminate the bacteria completely within 48 h. Further, against *S. dyesteriae* NICED, the compound **5a** took 72 h to inhibit the growth of the bacteria completely. The positive control ciprofloxacin (CIP) took 48 h to kill the tested bacteria completely except *S. dyesteriae* NICED. It completely exterminated *S. dyesteriae* within 72 h.

In conclusion, a simple, efficient and environmentally benign method was developed for the synthesis of 1,2-disubstituted benzimidazoles and 2-substituted benzothiazoles by using Al_2O_3–Fe_2O_3 nanomaterials having high surface area and high catalytic activity. The results based on disc diffusion method showed that, com-

pounds **3a**, **5a** and **5d** exhibited moderate to good antibacterial activity against all the tested bacterial stains. In addition, compound **5c** was selectively active against shigellosis causing bacteria *S. dysenteriae*. Moreover, the compounds **3a**, **3f** and **5a** displayed significant inhibitory activity compared to standard antibacterial drug ciprofloxacin against *V. cholerae, B. cereus* and *S. dysenteriae*, respectively. The time point assay results revealed that, the compounds **3a**, **3e**, **3f**, **3h** and **5b** demonstrated complete bactericidal activity within 24 h, whereas ciprofloxacin took 48 h to kill those bacteria completely. These antibacterial results indicated that structural factors, such as type of substitution in the ring and water solubility of the target compounds could further enhance their inhibitory activities.

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Supplementary data

Supplementary data (experimental informations and spectral data (^{1}H, ^{13}C NMR, ESI-MS and elemental analysis)) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.034.

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25. To a mixture of 1,2-phenylenediamine (0.54 g, 5 mmol) and aromatic/aliphatic aldehyde (10 mmol), catalytic amount (0.0108 g, 5 wt % of 1,2-phenylenediamine) of Al_2O_3–Fe_2O_3 nanocrystals was added using 5 mL of acetonitrile as solvent at room temperature under stirring. The reaction progress was monitored by TLC. After stirring for 10–45 min, under heating condition at 60 °C, the reaction mixture was cooled to room temperature and it was dissolved in ethanol (20 mL) and then poured into ice-water (40 mL). Ethyl acetate (50 mL) was added and the catalyst was separated out by filtration from the extraction mixture. The organic extract was dried over anhydrous sodium sulfate and excess of solvent was removed under reduced pressure so as to obtain the product. It was then purified by column chromatography over silica gel using hexanes/EtOAc as the eluting solvent system, yielding the pure products. An identical procedure for the synthesis of 2-substituted benzothiazoles was employed using 2-aminothiophenol (0.53 mL, 5 mmol) and aromatic/aliphatic aldehyde (5 mmol) in the presence of catalytic amount (5 wt % of 2-aminothiophenol) of Al_2O_3–Fe_2O_3 nanocrystals under heating condition (60 °C).
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29. The newly synthesized compounds which showed antibacterial activity in agar diffusion method were selected for further screening though quantitative assay at different time point. In this method, minimum time required to kill the bacteria was estimated by pour plate method at different time point. Mueller–Hinton broths (5 mL) were prepared in a test tube, sterilized and cooled to room temperature and incubated at room temperature for overnight to check the sterility of the media. A loop full of respective bacterial cultures (*B. cereus* MTCC 430, *S. aureus* MTCC 3160, *E. coli* MTCC 1610, *V. cholerae* MTCC 3904 and *S. dysenteriae* NICED) were inoculated and incubated at 37 °C for overnight, 100 μL of cultures were withdrawn and checked the bacterial density by standard pour plate method. Approximately 0.2 mg/mL concentrations of the synthesized compounds was added to each tube and incubated at 37 °C for up to 72 h. Every 24 h interval the fresh M–H broths were replenished. At different time interval 100 μL of samples were withdrawn and subjected to standard serial dilutions and plated on MHA plates by pour plate method. The plates were inverted and incubated at 37 °C for 18–24 h. Observed the colonies and counted the colonies using colony counter and estimated the bacterial density of the sample and expressed as colony forming unit (CFU)/ml. All the bacterial cultures without the addition of synthesized compounds were used as control sample. Ciprofloxacin was used as reference drug (positive control) in this study.