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Facile synthesis of *N*-substituted-2-(3,5-dinitrophenyl) benzimidazole derivatives for antimicrobial investigation

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***Abstract*—** Benzimidazole derivatives are crucial structural heterocyclic motifs found in diverse libraries of biologically active compounds which are therapeutically useful agents in drug discovery and medicinal research. 2-(3,5-Dinitrophenyl) benzimidazole, 1 was synthesized by [4+1]-cycloaddition of *o*-phenylenediamine with 3,5-dinitrobenzoic acid in catalytic amount of NH_4Cl. Compound 1 was subsequently utilized as precursor which upon reaction with some halogenated molecular entity furnished the targeted *N*-substituted-2-(3,5-dinitrophenyl) benzimidazole derivatives in improved yields after reaction optimization engagement. The progress of reaction was monitored with TLC and the chemical structures were confirmed by analytical data and spectroscopic means such as UV, IR, mass spectra, ^{1}H NMR, ^{13}C NMR and DEPT-135. The titled compounds were screened for their antimicrobial activity alongside with gentamicin standard drug, using agar diffusion method while the minimum inhibitory concentration (MIC) was carried out using serial dilution method. The result showed that the synthetic method used herein was successful for the preparation of the titled benzimidazole motifs in high yields and eco-friendly manner. The compounds showed broad spectrum of activity and most of them competed favourably with gentamicin and they exhibited highly promising antibacterial potential for future drug development. They are good candidates for further studies in term of SAR study and other pharmacological screenings essential in therapeutic research.

Keywords—Antibacterial, catalysis, cyclo-addition, benzimidazole, spectroscopy, chromatography

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Introduction

Benzimidazole derivatives are crucial structural scaffolds found in diverse libraries of biologically active compounds which are therapeutically useful agents in drug discovery and medicinal research [1]. Numerous compounds containing benzimidazole moieties have been reported to exhibit diverse biological and pharmacological properties which include, but not limited to, antitumor [2], anticancer [3], antimalarial [4], anti-inflammatory [5], antiepileptic [6], antitubercular [7], anti-HIV [8], antihypertensive [9], antimicrobial [10], anthelmintic [11], antioxidant [12] and anti-diabetic [13] activities. Conventionally, a drug is designated by its dominant or by its first recognized function; hence, benzimidazole nucleus is the core structure of several drugs such as bendamustine as anticancer; omeprazole as anti-ulcer; albendazole as anthelmintic, benomyl as antifungal; telmisartan as antihypertensive; astemizole as receptor antagonist drugs [14]. A major problem with antibiotics and other old existing drugs is that their overuse and improper use has led to the development of drug-resistant bacteria; thus, an increased number of infections with opportunistic bacteria, such as enterococci, coliforms, pseudomonas, and streptococci which are versatile and capable of becoming multidrug resistant in their mechanism of actions.

Materials and Method

General Condition – All the reagents were purchased from Sigma-Aldrich, were made available by Department of Chemistry, Covenant University and were used directly without further purification. Infrared spectra were recorded on a Schimadzu Spectrometer. The Ultraviolet spectra were run on a Genesys Spectrometer using tetrahydrofuran THF. The ^{1}H-NMR of the compounds were run in DMSO-d_6 on NMR Bruker DPX 400 spectrometer operating at 400 MHz. All compounds were routinely checked by TLC on silica gel G plates using $CHCl_3$:CH_3OH (9:1, v/v) solvent system. The solvents were evaporated under reduced pressure using IKA® RV 10 Rotary evaporator. Where necessary, column chromatographic purifications were carried out on Merck silica gel F (Mesh 200-300). Melting points were determined in open capillary tubes on a Stuart melting point apparatus and were uncorrected.

Synthesis – 2-(3,5-dinitrophenyl)-1*H*-benzimidazole as precursor 1. *o*-Phenylene diamine (15.00 g, 140.00 mmol) was weighed into 250 ml round bottom flask, 150 ml of ethanol was added. The mixture was stirred for 5 minutes under the influence of a magnetic stirrer after which 3,5-dinitrobenzoic acid (29.70 g, 140.00 mmol) was added to the partially soluble mixture followed by the addition of a catalytic amount of NH_4Cl (0.75 g, 14.00 mmol). The resulting mixture was heated under reflux with the influence of a magnetic stirrer under high temperature for 2 h. The progress of the reaction was monitored by TLC and the reaction was terminated after completion and allowed to cool at room temperature. The solvent was removed by

rotary evaporation and cold water was added to the residue, stirred with stirring rod and filtered by suction to afford 2-(3,5-dinitrophenyl)-1*H*-benzimidazole as precursor, **1** (90.07%). ^{1}H-NMR (400 MHz, DMSOd$_6$) δ_H: 8.85 (s, 1H, Ar-H), 8.64 (s, 2H, Ar-H), 7.96-7.94 (m, 1H, Ar-H), 7.74-7.72 (t, *J* = 7.58 Hz, 1H, Ar-H), 7.46-7.44 (d, *J* = 8.00 Hz, 1H, Ar-H), 7.42-7.40 (d, *J* = 8.00 Hz, 1H, Ar-H). λ_{max} in nm (log ε_{max}): 218 (4.6522), 248 (4.6365). FT-IR (ν_{max} in cm^{-1}): 3349 (N-H), 3172 (C-H aromatic), 3101 (C-H aromatic), 1606 (C=C), 1572 (C=N), 1543 (NO_2 asym.), 1344 (NO_2 sym.), 1076 (C-N), 916 (N-O), 721 (Ar-H).

Synthesis – *N*-Substituted-2-(3,5-dinitrophenyl)-1*H*-benzimidazole. 2a-f. 2-(3,5-Dinitropheny l)-1*H*-benzimidazole, **1** (4.00 g, 13.60 mmol) was weighed into round bottom flask followed by the addition of 20 ml of tetrahydrofuran (THF) under continuous stirring with the aid of a magnetic stirrer at room temperature. The flask was placed inside ice bath to achieve temperature of 0-5 ^{o}C followed by the addition of corresponding substrate (13.60 mmol) drop wisely to the stirring solution and the reaction was maintained on ice bath for additional 15 minutes after which the ice bath was removed and the resulting mixture was stirred at room temperature for 24 h. The progress of the reaction was monitored by TLC and upon reaction completion, the mixture was concentrated using rotary evaporator to remove the solvent. Cold water was added to the resulting mass and then filtered by suction. The solid obtained was air-dried to afford *N*-substituted-2-(3,5-dinitrophenyl)-1*H*-benzimidazoles **2a-f.**

1-(2-(3,5-dinitrophenyl)-1*H*-benzo[*d*]imidazole-1-yl)ethanone, **2a.** ^{1}H-NMR (400 MHz, DMSOd$_6$) δ_H: 8.85 (s, 1H, Ar-H), 8.64 (s, 2H, Ar-H), 7.96-7.94 (m, 1H, Ar-H), 7.74-7.72 (t, *J* = 7.56 Hz, 1H, Ar-H), 7.46-7.44 (d, *J* = 8.00 Hz, 1H, Ar-H), 7.42-7.40 (d, *J* = 8.00 Hz, 1H, Ar-H), 2.59 (s, 3H, CH_3-CO). λ_{max} in nm (log ε_{max}): 218 (4.3729), 248 (4.3050). FT-IR (ν_{max} in cm^{-1}): 3106 (C-H aromatic), 2854 (C-H aliphatic), 1699 (C=O amide), 1622 (C=C), 1580 (C=N), 1541 (NO_2 asym.), 1346 (NO_2 sym.), 1083 (C-N), 927 (N-O), 719 (Ar-H).

2-(3,5-dinitrophenyl)-1-tosyl-*H*-benzo[*d*]imidazole,2c. ^{1}H-NMR (400 MHz, DMSOd$_6$) δ_H: 8.84 (s, 1H, Ar-H), 8.60 (s, 2H, Ar-H), 7.95-7.93 (m, 1H, Ar-H), 7.75-7.73 (t, *J* = 7.54 Hz, 1H, Ar-H), 7.44-7.40 (m, 2H, Ar-H), 7.17-7.15 (d, *J* = 8.00 Hz, 2H, Ar-H), 6.92-6.90 (d, *J* = 8.00 Hz, 2H, Ar-H), 2.60 (s, 3H, CH_3-Ar). λ_{max} in nm (log ε_{max}): 242 (4.6138). FT-IR (ν_{max} in cm^{-1}): 3105 (C-H aromatic), 2852 (C-H aliphatic), 1620 (C=C), 1575 (C=N), 1540 (NO_2 asym.), 1377 (SO_2), 1345 (NO_2 sym.), 1278 (C=N bending), 1187 (SO_2), 1085 (C-N), 927 (N-O), 724 (Ar-H).

Result and Discussion

Benzimidazole is an important pharmacophore which was included in several biologically active compounds resulted in the development of several classes of drugs. Owing to wide biological application of benzimidazole, we have herein embarked upon synthesis of this highly relevant heterocycle for the sake of their antimicrobial investigation for possible future drug development. The synthesis of 2-(3,5-dinitrophenyl)benzimidazole **1** which was used as the reactive intermediate in this study was as depicted in Scheme 1a. It was achieved by ecofriendly synthetic approach using NH_4Cl catalyzed coupling of o-phenylenediamine with 3,5-dinitrobenzoic acid according to the procedure described by Rithe et al. [15]. Secondly, the reactive intermediate 1 was reacted with six different electrophile-donating substrates in a basified environment from ice-bath to room temperature to afford the targeted *N*-substituted-2-(3,5-dinitrophenyl)benzimidazole motifs final product **2a-f**. (Scheme 1b). The result of the physicochemical parameter (Table 1) showed that the products were obtained in good to excellent yields with compound **2e** having the lowest yield (61.04%) while compound **2c** was reported to have the highest yield (98.77%). Compound 2c had the lowest and harp melting point (112 ^{o}C) while compound **2b, 2d, 2e** and **2f** did not melt at 300 ^{o}C. The virtue screening unveiled the reactive intermediate **1** to be yellow in colour while compounds **2a** and **2c** were brown, compounds **2b, 2e** and **2f** were gray and only compound **1d** was black in colour. The % calculated for C, H, N for the compounds were in agreement with the % found with not more ±0.20 difference (Table 1).

<<<<<Scheme 1>>>>>

<<<<<Table 1>>>>>

From the spectroscopic point of view, the ^{1}H-NMR spectra of the compounds were run in DMSO-d$_6$ using 400 MHz machine capacity. All the aromatic protons resonated downfield from δ 8.90-6.90 ppm while CH_3 of **1a** and **1c** were seen upfield as 3H singlet at δ 2.60-2.59 ppm while CH_2 of **1d** appeared as 2H singlet at δ 3.56 ppm. The uv transition was run in THF for the precursor **1** and final compounds **2a-f** wherein the lowest wavelengths observed at 212 to 218 nm, were as a result of the π→π* transition of C=C which depicted the presence of benzene ring in them. Bathochromic shifts led to the presence of other peaks at higher wavelengths ranging from 235 to 470 nm. Some of these were as a result of π→n transition which may be ascribed to the auxochromic C=N group; characteristic of K bands of C=N functional group [16]. The infrared spectra of the compounds **2a-f** showed absorption bands due to the stretching vibrations of C-H aromatic, C=C, C=N and NO_2 (asym.) at 3106-3050, 1622-1600, 1580-1575 and 1543-1540 cm^{-1} respectively. An additional band was noticed in **2a** at 1699 cm^{-1} which depicted the presence of C=O of amide while two bands at 1377-1375 and 1187-1185 cm^{-1} noticeable in products **2b** and **2c** represented the two SO_2 bands peculiar to those two compounds alone.

<<<<<Table 2>>>>>

The result of antimicrobial screening (sensitivity testing) using agar diffusion method [17] at concentration of 100 mg/ml on the four organisms (Staphylococcus aureus, *Bacillus lichenformis*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) with zones of inhibition (Z.O.I) in millimeter (mm) as indicated in Table 2. The use of gentamicin as clinical standard is due to the fact that gentamycin at low concentration is able to inhibit the growth of bacterial and also prevents the initiation of protein synthesis and leads to

death of microbial cell (Voet and Voet, 2004). All the compounds showed growth inhibition potential against the four organisms used except in the case *Bacillus lichenformis* and *Pseudomonas aeruginosa* which showed resistance to **2d, 2e, 2f** and Proteus vulgaris which showed resistance to compounds **2a** and **2d**. The sulfonamide-based benzimidazole **2b** emerged as the most active among all the series with its Z.O.I. ranged from 18 mm to 30 mm at the prepared concentration.

Conclusion

The intermediate precursor **1** was successfully achieved in high yields using NH_4Cl mediated approach while the functionalized 2-substituted benzimidazole **2a-f** were obtained via easier work-up and highly economical and ecofriendly strategy in Na_2CO_3. The results showed that this skeletal framework exhibited marked potency as antimicrobial agents. The most active antibacterial agent was **2b**.

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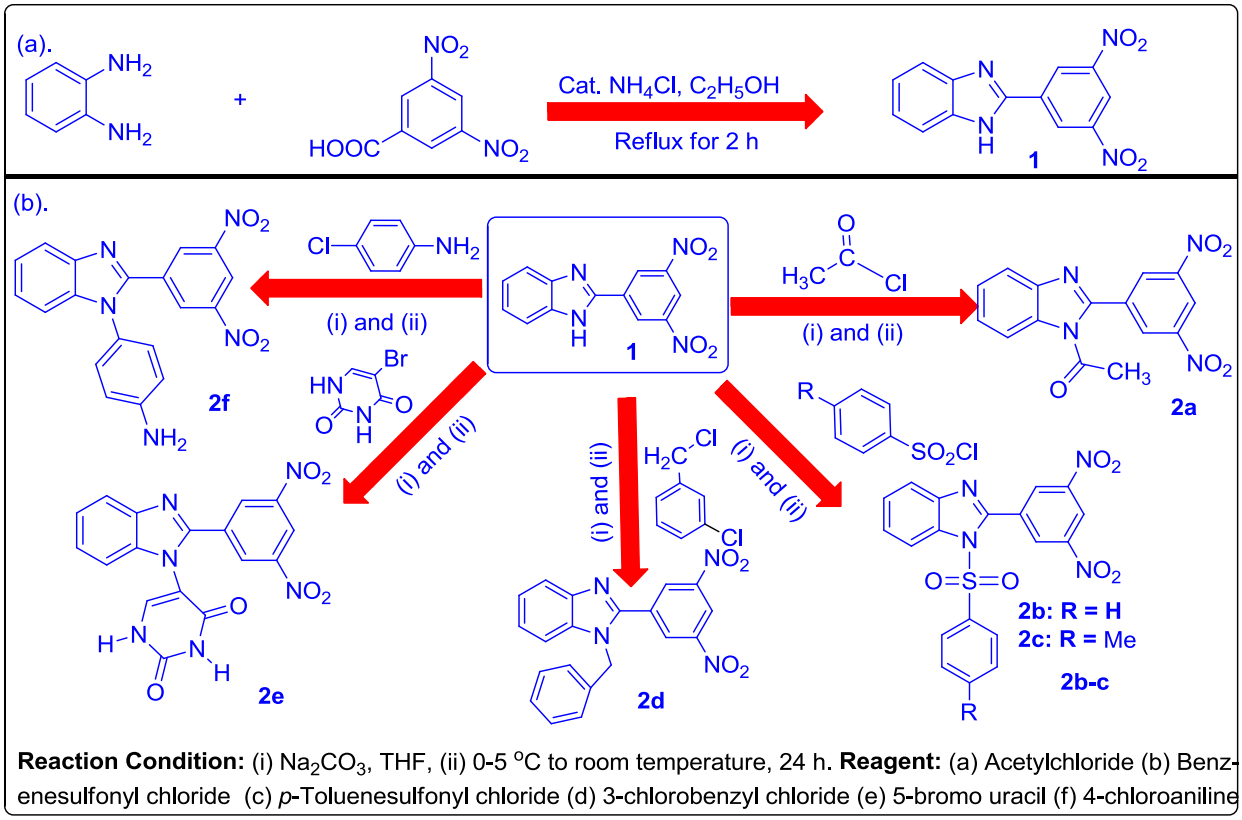

Reaction Condition: (i) Na_2CO_3, THF, (ii) 0-5 °C to room temperature, 24 h. **Reagent:** (a) Acetylchloride (b) Benzenesulfonyl chloride (c) *p*-Toluenesulfonyl chloride (d) 3-chlorobenzyl chloride (e) 5-bromo uracil (f) 4-chloroaniline

Scheme 1: (a). Synthetic pathway to Precursor **1** (b). Synthesis of the Final Benzimidazole Products **2a-f**

Table 1: The Result of the Physicochemical Properties of the Synthesized Compounds

Comp No	Mol. Form. (Mol. Wt.)	Mol. Wt.	Melting Pt. (°C)	Yield (%)	Sample Colour	Elemental Analysis %Calcd. (% Found)		
						C	H	N
1	$C_{13}H_8N_4O_4$	284.22	177-179	90.07	Yellow	55.13(54.98)	2.49(2.54)	19.78(19.92)
2a	$C_{15}H_{10}N_4O_5$	326.26	250-252	72.58	Brown	55.22(55.13)	3.09(2.89)	17.17(17.08)
2b	$C_{19}H_{12}N_4O_6S$	434.39	>300	62.77	Gray	53.77(53.95)	2.85(3.03)	13.20(13.31)
2c	$C_{20}H_{14}N_4O_6S$	438.41	112(s)	98.77	Brown	54.79(54.62)	3.22(3.16)	12.78(12.94)
2d	$C_{20}H_{13}N_4O_4Cl$	408.79	>300	71.16	Black	58.76(58.88)	3.21(3.32)	13.71(13.89)
2e	$C_{17}H_{10}N_6O_6$	394.30	>300	61.04	Gray	51.78(51.97)	2.56(2.69)	21.31(21.51)
2f	$C_{19}H_{13}N_5O_4$	375.34	>300	66.85	Gray	60.80(61.00)	3.49(3.68)	18.66(18.84)

Comp No = Compound No; Mol. Form. = Molecular Formular; Mol. Wt. = Molecular Weight; (s) = Sharp.

Table 2: Result of antibacterial sensitivity testing with zones of inhibition in (mm)

Bacteria → / Comp. No↓	*S. aureus*	*B. lichenformis*	*P. vulgaris*	*P. aeruginosa*
1	15.00 ± 0.08	20.00 ± 0.08	30.00 ± 0.12	28.00 ± 0.09
2a	20.00 ± 0.10	24.00 ± 0.09	R	25.00 ± 0.08
2b	30.00 ± 0.10	18.00 ± 0.08	20.00 ± 0.08	25.00 ± 0.08
2c	25.00 ± 0.10	15.00 ± 0.08	20.00 ± 0.08	25.00 ± 0.08
2d	30.00 ± 0.09	R	R	R

2e	30.00 ± 0.10	R	20.00 ± 0.08	R
2f	30.00 ± 0.12	R	23.00 ± 0.08	R

S. aureus = *Staphylococcus aureus* (G^+), *B. lichenformis* = *Bacillus lichenformis* (G^+). *P. vulgaris* = *Proteus vulgaris* (G^-). *P. aeruginosa* = *Pseudomonas aeruginosa* (G^-), Gtm. = Gentamicin. G^+ = Gram positive, G^- = Gram negative. Z.O.I. = Zone of Inhibition in (mm). R = Resistant. N.D. = Not Determined.

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