Room-Temperature Synthesis of Aryl-Substituted Benzimidazoles and their Toxicity against *Artemia salina*

Jerson E. Lapetaje and Evelyn C. Creencia
College of Science and Mathematics
Mindanao State University – Iligan Institute of Technology
Iligan City, 9200 Philippines
**jerson.lapetaje@g.msuiit.edu.ph*

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Abstract

Benzimidazoles are known to display pharmacological uses with a wide range of noticeable importance, such as antifungal, antiviral, antihypertensive, and antimicrobial. Thus, successful syntheses of benzimidazole containing compounds have been the primary goal of researchers and organic chemists in the medical industry and drug discovery. 'Green chemistry' approach to developing drug leads has been widely encouraged since the past decades. In this study, the 'green chemistry' approach in synthesizing benzimidazole derivatives was successfully utilized to yield compounds at room temperature using ferric sulfate ($Fe_2(SO_4)_3/SiO_2$) as the catalyst and then subjected to toxicity assay against Artemia salina. Fourier transform-infrared spectroscopy, nuclear magnetic resonance, and mass spectral analyses of the synthesized compounds confirmed the structures of the synthesized benzimidazole derivatives. Moreover, the method gave satisfactory yields of about 22.62- 51.94%. The toxicity results revealed a wide range varied from mild to highly toxic (<10.00 ppm - 968.72 ppm) at chronic levels, suggesting a potential application of synthesized compounds in the pharmacological industry.

***Keywords**:* room-temperature synthesis, benzimidazoles, $Fe_2(SO_4)_3/SiO_2$-catalyzed, *Artemia salina*

1. Introduction

Heterocyclic compounds play a vital role in biological systems and are of immense importance in the fields of pharmaceuticals and agrochemicals (Basu and Mandal, 2015). Among the important nitrogen-containing heterocycles are benzimidazoles, an imidazole ring fused with a benzene ring. Different researches have shown that benzimidazole-containing compounds could exhibit wide range of biological activity such as antifungal (Bai *et al.*, 2013),

antiviral (Zou *et al.*, 1996), antimicrobial (Padalkar *et al.*, 2014), proton pump inhibitors (Murray *et al.*, 2012), anticancer, antiparasitic and insecticidal (Gurvinder, 2013), just to name a few. Thus, the syntheses of these biologically active compounds have been significant for the past decade. However, the synthesis of these diverse bioactive compounds has caused quite a menace to the environment due to the use of hazardous chemicals and solvents in the reaction and purification processes. Therefore, it is a must for researchers to provide a safer and greener approach in attaining these processes that is under the pillars of "green chemistry". One of these approaches is the use of prominent catalysts like iron. Iron has been used variously not only in the industry but also in pharmacology. Its great potential as an abundant and inexpensive catalyst has paved its way in the synthesis of organic compounds. Researches have indicated that iron is a good catalyst for the synthesis of tetrahydroquinoline (López and González, 2015), indole-derivatives (Dar *et al.*, 2013). Iron also acts as a catalyst in decreasing free fatty acid content in vegetable oil (Đokić *et al.*, 2012). One research (Paul and Basu, 2012) used iron ($Fe_2(SO_4)_3/SiO_2$) as a catalyst for the synthesis of 1,2-disubstituted benzimidazoles with high yields under benign conditions. These studies provide a good background in using iron in the form of $Fe_2(SO_4)_3$ in SiO_2 for the effective and selective synthesis of benzimidazole derivatives.

Hence, this study aims to synthesize benzimidazole derivatives under benign conditions using iron in the form of $Fe_2(SO_4)_3$ in SiO_2 as a catalyst. The study further evaluated their toxicity activity using brine shrimp lethality test (BSLT) for preliminary screening of biological activity.

2. Methodology

2.1 Optimization of $Fe_2(SO_4)_3/SiO_2$ Catalyst

O-phenylenediamine (1) and 4-fluorobenzaldehyde (4c) were used for the optimization of the %w/w composition of $Fe_2(SO4)_3/SiO_2$ by obtaining the % crude yields at different composition (10, 40, and 70%). Reactants were selected based on their availability in the laboratory. About 1 mmol (124 mg) of o-phenylenediamine, 1 mmol (108 µL) of 4-fluorobenzaldehyde, 5 mL ethanol, and 0.100-gram (10% w/w, 40% w/w, and 70%w/w) $Fe_2(SO4)_3/SiO_2$ were placed separately in a 30-mL Erlenmeyer flask. The solution was magnetically stirred for an hour under room temperature. The progress of the reaction was monitored using thin-layer chromatography (TLC). Standard

purification method was employed where the reaction mixture was then filtered to remove the catalyst. The resulting filtrate was added with a pinch of activated charcoal and swirled for about 5 min. The activated charcoal was then filtered. The filtrate was added to 100 mL water to allow precipitation. Reaction mixtures that resulted in colloidal precipitate were added with 1 g of NaCl to induce the formation of a larger precipitate via the salting-out effect. The precipitate was then vacuum-filtered, vacuum-dried, and monitored for its purity using TLC (under 250-nm UV light). Further purification of other products that showed faded spots/impurities in their TLC profiles was executed using gravity column chromatography and/or recrystallization process. Method for optimization and all reactions were carried out in triplicates. The percent crude yield from different % w/w composition (10, 40, and 70 %) of $Fe_2(SO4)_3/SiO_2$ were then calculated.

2.2 General Procedure for the Synthesis of Aryl-Benzimidazoles

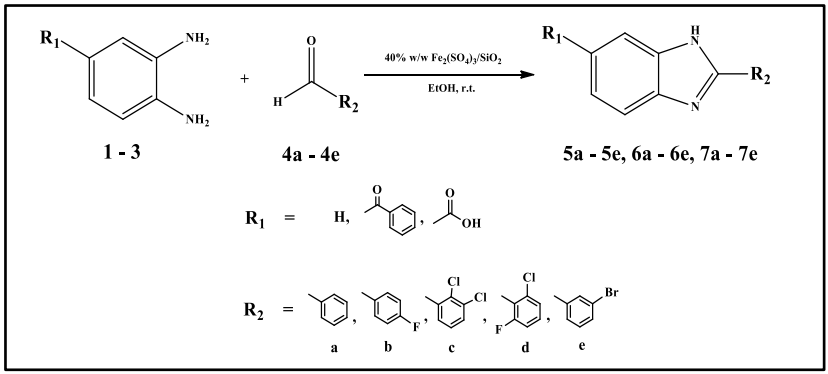
In a 30-mL Erlenmeyer flask, 1 mmol of o-phenylenediamine derivative, 1 mmol aryl aldehyde, 5 mL ethanol, and 0.100 g 40 % w/w $Fe_2(SO_4)_3/SiO_2$ were placed and magnetically stirred for 1 hour (h) under room temperature conditions (Scheme 1). The progress of the reaction was then monitored using TLC. Standard purification method was employed following the same method stated in the optimization of the catalyst. Consistent with the preceding section, further purification of other products that showed faded spots in their TLC profiles was performed using gravity column chromatography and recrystallization process.

2.3 FTIR-ATR Analysis

About 3 mg of the solid product and the reactants were placed in the attenuated total reflectance (ATR) smart accessory of the Nicolet *i*S50 Fourier transform-infrared spectroscopy (FT-IR) and the infrared (IR) spectra were obtained for the pure products and the reactants. IR data of the samples were then compared for elucidation purposes.

2.4 Nuclear Magnetic Resonance Analysis

About 30 mg of each of the sample products were sent to the Nuclear Magnetic Resonance (NMR) Facility of the Institute of Chemistry, College of Science, University of the Philippines- Diliman, Quezon City for the ^{1}H NMR, ^{13}C NMR, and correlation spectroscopy analysis of the synthesized products, using Agilent 500 MHz NMR spectrometer.



Scheme 1. General reaction scheme for the synthesis of aryl-substituted benzimidazoles

2.5 Mass Spectroscopy Analysis

Base line data for the products molecular weights were determined using the Shimadzu Liquid Chromatography – Mass Spectrometry single quadrupole (LCMS) 2020 instrument. 10 ppm solution of the synthesized compounds were prepared and injected into the LCMS 2020 instrument and analyzed using the following parameters: (1) method: flow injection; (2) flow rate: 0.2 mL/min; (3) injection volume: 1 µL; (4) solvent system: methanol with 0.1% formic acid; (5) ionization interface: electrospray ionization "ESI" (+ scan and – scan); (6) desolvation line (DL) temperature: 250 °C; (7) nebulizing gas flow: 1.5 L/min; (8) heat block: 250 °C; (9) drying gas flow: 15 L/min; (10) mass-to-charge ratio (m/z): 100 – 800; (11) scan speed: 3750; (12) threshold: 0; (13) event time: 0.2 s.

2.6 Brine Shrimp Lethality Test (BSLT)

BSLT is a widely utilized and accepted bioassay for the determination and characterization of potentially bioactive compounds. For the past years, many researchers conducted characterization of bioactive properties of a number of compounds and plant extracts using the BSLT. This is due to the reliability of BSLT's results, simplicity in application, cost efficiency, and low toxin concentration administration in performing the bioassay. Brine shrimp had been previously utilized in various bioassay systems. Among these applications were the analysis of pesticide residues (Tarpley, 1958), mycotoxins (Brown, 1969), stream pollutants (Hood, 1960), and anesthetics (Robinson, 1965), to name a few.

Stock solutions of 20,000 ppm of the synthesized products were prepared by dissolving 30 mg of each product in 1.5 mL Dimethyl sulfoxide (DMSO). Different concentrations of the products were prepared (10, 100, 500, and 1000 ppm) by obtaining aliquots of 2.5, 25, 125, and 250 µL from the stock solution and delivered into 10-mL test tubes. From the incubation vessel, 10 hatched brine shrimp *A. salina* were carefully delivered into the test tubes containing the 2.5, 25, 125, and 250 µL solution of the products, and then diluted to 5 mL total volume to achieve 10, 100, 500, and 1000 ppm concentrations, respectively. The solution containing the brine shrimp *A. salina* was monitored at 6 and 24 h for data collection of the number of live and dead test animals, which were then used for the determination of acute and chronic toxicity levels known as the lethal concentration at 50% mortality (LC_{50}). Obtained data were subjected to Reed-Muench statistical analysis to acquire a correlation of the toxicity and concentration of the products.

2.7 Catalyst Preparation

Different % w/w composition (10, 40, and 70%) of $Fe_2(SO_4)_3/SiO_2$ catalyst were prepared by grinding 0.200, 0.800, and 1.400 g of $Fe_2(SO_4)_3 \bullet xH_2O$ with 1.800, 1.200, and 0.600 g of 200 mesh SiO_2 for 45 min to 1 h until a uniform appearance of the catalyst was attained.

2.8 Compound Profile

Compound (5a) ($C_{13}H_{10}N_2$): IUPAC name - 2-phenyl-1H-benzo[d]imidazole; Beige solid; mp 289-298 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3058, 1622-1590, 1587, 1445, 1318; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.22 - 8.20 (9H, m, Ar-H), 12.97 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 111.57, 122.15, 122.99, 126.70, 129.32, 130.13, 135.43, 143.87, 151.68 ppm; m/z (ESI Scan +) = 195.0.

Compound (5b) ($C_{13}H_8Cl_2N_2$): IUPAC name - 2-(2,3-dichlorophenyl)-1H-benzo[d]imidazole; Beige crystal; mp 237-238 ºC; IR (ATR) (v_{max}, cm^{-1}): 3100, 1532.26, 1420.70, 1320.41 – 1277.65, 1115.15 – 707.49; ^{1}H NMR (500 MHz, DMSO-d6 and $CDCl_3$ 1:1) δ = 7.18 -7.79 (7H, m, Ar-H), 12.66 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 122.72, 128.16, 130.83, 131.65, 132.78, 133.50, 148.99 ppm; m/z (ESI Scan +) = 262.9.

Compound (5c) ($C_{13}H_9FN_2$): IUPAC name - 2-(4-fluorophenyl)-1H-benzo[d]imidazole; Beige crystal; mp 254-255 ºC ; IR (ATR) (v_{max},

cm^{-1}): 3052.54, 1603.33, 1497.04 – 1432.15, 1275.88 – 1226.77, 966.70, 834.15, 742.76; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.22 - 8.25 (8H, m, Ar-H), 12.96 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 111.77, 116.56, 121.72, 122.58, 129.16, 144.21, 150.83, 162.52 ppm; m/z (ESI Scan +) = 213.0.

Compound (5d) ($C_{13}H_8ClFN_2$): IUPAC name - 2-(2-chloro-6-fluorophenyl)-1H-benzo[d]imidazole; Colorless crystal; mp 216-217 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3052.54, 1603.33, 1497.04 – 1432.15, 1275.88 – 1226.77, 866.70, 834.15, 742.76; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.20-7.67 (7H, m, Ar-H), 12.96 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 115.46, 120.06, 123.35, 126.31, 133.08, 134.74, 143.98, 160.13, 162.12 ppm; m/z (ESI Scan +) = 247.0.

Compound (5e) ($C_{13}H_9BrN_2$): IUPAC name - 2-(3-bromophenyl)-1H-benzo[d]imidazole; Beige solid; mp 247-248 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3100, 1563.94, 1438.00 – 1399.77, 1228.79 – 1360.68, 1072.45, 893.13, 741.50, 679.96; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.20 – 8.37 (8H, m, Ar-H), 13.05 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 122.72, 125.81, 129.32, 131.63, 132.86, 145.00, 150.05 ppm; m/z (ESI Scan +) = 272.9.

Compound (6a) ($C_{20}H_{14}N_2O$): IUPAC name - phenyl(2-phenyl-1H-benzo[d]imidazol-6-yl)methanone; Pale yellow solid; mp 185-186 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3374.60, 1612.23-1578.20, 1469.15 – 1482.98, 1325.67-1282.62, 1104.70, 912.44, 832.48, 702.95; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.40-7.92 (11H, m, Ar-H), 8.17 (2H, bs, Ar-H), 13.44 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 124.63, 127.21, 128.87, 129.54, 129.92, 130.96, 132.54, 138.53, 154.5, 196.07 ppm; m/z (ESI Scan +) = 299.1.

Compound (6b) ($C_{20}H_{12}Cl_2N_2O$): IUPAC name - (2-(2,3-dichlorophenyl)-1H-benzo[d]imidazol-6-yl)(phenyl)methanone; Pale yellow solid; mp 132-133 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3065.55, 1649.94 – 1624.11, 1446.19-1414.63, 1317.06,1242.45, 984.18, 886.61, 786.17; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.54 – 8.00 (11H, m, Ar-H), 13.28 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 124.68, 128.92, 129.07, 129.95, 131.36, 131.78, 132.33, 132.33, 132.57, 132.65, 138.43, 151.80, 196.11 ppm; m/z (ESI Scan +) = 367.0.

Compound (6c) ($C_{20}H_{13}FN_2O$): IUPAC name - (2-(4-fluorophenyl)-1H-benzo[d]imidazol-6-yl)(phenyl)methanone; Beige crystal; mp 242-

243 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3232.60, 1640.37-1594.54, 1488.72-1420.44, 1314.27-1227.33, 949.84, 897.37, 840.57, 708.46; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.23 – 8.21 (12H, m, Ar-H), 13.07 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 116.07, 116.25, 124.61, 128.41, 129.32, 129.78, 131.49, 132.09, 138.54, 162.85, 164.83, 195.98 ppm; m/z (ESI Scan +) = 317.1.

Compound (6d) ($C_{20}H_{12}ClFN_2O$): IUPAC name - (2-(2-chloro-6-fluorophenyl)-1H-benzo[d]imidazol-6-yl)(phenyl)methanone; Beige solid; mp 209 - 211 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3070.42, 1652.81 – 1575.33, 1451.93, 1317.06 – 1276.88, 906.70, 786.17, 702.95; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.45 – 8.03 (11H, m, Ar-H), 13.40 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 115.51, 119.83, 126.42, 128.92, 130.00, 132.26, 133.54, 134.63, 138.39, 146.71, 160.05, 162.05, 196.14 ppm; m/z (ESI Scan +) = 351.0.

Compound (6e) ($C_{20}H_{13}BrN_2O$): IUPAC name - (2-(3-bromophenyl)-1H-benzo[d]imidazol-6-yl)(phenyl)methanone; White solid; mp 220 - 221 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3292.72, 1639.00 – 1528.64, 1439.64, 1351.75 – 1266.57, 1099.27, 980.71, 822.31, 708.56; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.41 – 8.36 (12H, m, Ar-H), 13.18 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 122.79, 125.82, 128.43, 129.72, 129.77, 131.00, 132.14, 133.13, 138.46, 152.02, 195.94 ppm; m/z (ESI Scan +) = 379.0.

Compound (7a) ($C_{14}H_{10}N_2O_2$): IUPAC name - 2-phenyl-1H-benzo[d]imidazole-6-carboxylic acid; Beige solid; mp 208-210 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3064.68, 1684.37-1618.37, 1443.32-1403.15, 1271.14 – 1225.23, 829.22, 774.69, 757.48, 649.34; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.00 – 8.21 (13H, m, Ar-H), 13.08 (1H, s, N-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 47.80, 111.50, 113.50, 119.46, 124.08, 125.54, 126.37, 126.53, 129.30, 129.41, 129.50, 130.01, 163.03, 137.04, 146.38, 156.44, 168.22 ppm; m/z (ESI Scan +) = 329.1.

Compound (7b) ($C_{14}H_8Cl_2N_2O_2$): IUPAC name - 2-(2,3-dichlorophenyl)-1H-benzo[d]imidazole-6-carboxylic acid; Brown solid; mp 322-323 ºC ; IR (ATR) (v_{max}, cm^{-1}): 3276.08, 1659.66-1614.05, 1445.85-1411.64, 1311.6-1200.68, 915.60, 790.16, 773.06, 775.95, 621.96; ^{1}H NMR (500 MHz, DMSO-d6) δ = 7.41-8.27 (6H, m, Ar-H), 12.96 (1H, s, N-H), 15.26 (1H, s, O-H) ppm; ^{13}C NMR (126 MHz, DMSO-

d6) δ = 124.17, 125.38, 128.27, 130.72, 130.91, 132.03, 132.27, 133.58, 151.17, 168.35 ppm; m/z (ESI Scan +) = 307.0.

Compound (7c) ($C_{14}H_9FN_2O_2$): IUPAC name - 2-(4-fluorophenyl)-1H-benzo[d]imidazole-6-carboxylic acid; Beige solid; mp 320 - 322 ^oC ; IR (ATR) (v_{max}, cm^{-1}): 3053.71, 1656.81 – 1628.30, 1514.27 – 1420.19, 1291.91 – 1254.24, 1166.47, 929.85, 850.03, 830.07, 778.76; ^1H NMR (500 MHz, DMSO-d6) δ = 7.40-8.27 (7H, m, Ar-H), 12.85 (1H, s, N-H), 13.24 (1H, s, O-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 111.58, 116.48, 116.65, 123.94, 125.08, 129.49, 129.56, 143.94, 147.49, 153.09, 162.82, 168.30 ppm; m/z (ESI Scan +) = 257.1.

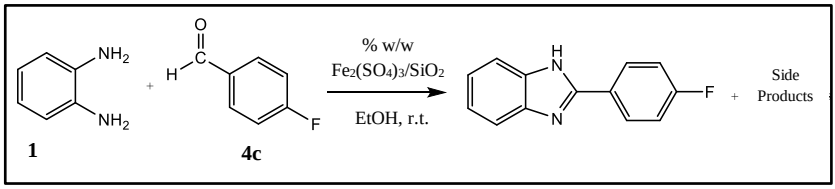
Compound (7d) ($C_{13}H_{10}N_2$): IUPAC name - 2-(2-chloro-6-fluorophenyl)-1H-benzo[d]imidazole-6-carboxylic acid; Beige solid; mp 324-326 ^oC ; IR (ATR) (v_{max}, cm^{-1}): 3133.54, 1653.96 – 1571.29, 1454.40 – 1414.49, 1314.71 – 1209.23, 901.34,781.61, 741.70, 710.34; ^1H NMR (500 MHz, DMSO-d6) δ = 7.47 – 8.26 (6H, m, Ar-H), 12.85 (1H, s, N-H), 13.31 (1H, s, O-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 111.91, 115.36, 121.57, 123.44, 124.73, 125.03, 133.37, 133.45, 137.86, 146.02, 146.83, 160.06, 168.24 ppm; m/z (ESI Scan +) = 291.0.

Compound (7e) ($C_{14}H_9BrN_2O_2$): IUPAC name - 2-(3-bromophenyl)-1H-benzo[d]imidazole-6-carboxylic acid; Beige solid; mp 270 – 274 ^oC ; IR (ATR) (v_{max}, cm^{-1}): 3085.07, 1668.21 – 1617.75, 1434.45, 1323.26 – 1303.31, 969.76, 795.86, 767.35, 747.40, 721.74, 678.98, ^1H NMR (500 MHz, DMSO-d6) δ = 7.51 – 8.37 (7H, m, Ar-H), 12.87 (1H, s, N-H), 13.28 (1H, s, O-H) ppm; ^{13}C NMR (126 MHz, DMSO-d6) δ = 122.75, 124.27, 125.37, 126.11, 129.59, 131.66, 132.26, 133.39, 140.12, 147.02, 152.58, 168.24 ppm; m/z (ESI Scan +) = 316.9.

3. Results and Discussion

3.1 Catalyst Optimization

Optimization of the catalyst was done by separately mixing 10, 40, and 70% w/w composition of $Fe_2(SO_4)_3/SiO_2$ with o-phenylenediamine (1) and 4-fluorobenzaldehyde (4c) as the model reaction (Scheme 2).



Scheme 2. Model reaction for catalyst optimization

The optimization of the catalyst was based on the evaluation of crude yields obtained from the model reaction for each % w/w catalyst composition. After synthesis and partial purification of the products, percent yields were calculated. The melting point was then compared to literature values (Dabhade *et al.*, 2009; Weires *et al.*, 2012) as presented in Table 1. IR spectra of the products were also obtained using FT-IR Nicolet *i*S50 with ATR accessory to observe the functional groups present (Figure 1).

Table 1. Melting point comparison of Product 5c.1, 5c.2, and 5c.3 with literature values

%w/w $Fe_2(SO_4)_3/SiO_2$	Product Code	Color of Product	Melting point Experimental Value (°C)	Melting Point Literature Value (°C)
10	5c.1	Beige	254-255	249-251
40	5c.2	Beige	253-254	249-251
70	5c.3	Beige	254-255	249-251

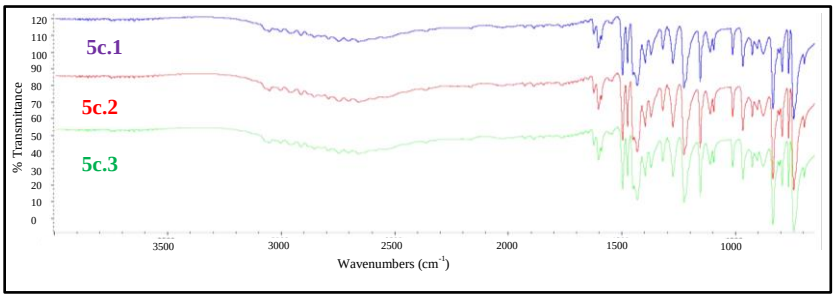


Figure 1. IR spectra of 5c.1, 5c.2, and 5c.3 showing the same peaks in the finger print region and far IR region

Analysis of the spectra for 5c.1, 5c.2, and 5c.3 showed prominent peaks at around 1226.77 cm^{-1} and 1623.25 cm^{-1} – indicating an imidazole group, which is prominently given by an aromatic C=N stretching. IR data of synthesized compounds were also compared to literature values (using KBr disk) shown in Table 2. Data suggests that the compounds are benzimidazole compounds confirming that the catalyst can facilitate condensation reactions. Moreover, compounds were identified to be 2-(4-fluorophenyl)-1*H*-benzimidazole. Thus, percent yields for the synthesized products can be compared. Percent yields for the synthesized products using 10, 40, and 70% w/w $Fe_2(SO_4)_3/SiO_2$ were compared and gave percent yield values of 47.17, 54.22, and 55.66%, respectively. However, it was observed that as % w/w exceeds 40 % (70 %w/w $Fe_2(SO_4)_3/SiO_2$), only a minor rise in the yield was observed. This indicates that an increase in the ratio of $Fe_2(SO_4)_3$ with SiO_2 beyond 40% does not elicit a significant increase in yield; further suggesting that a 40% w/w composition of the catalyst is sufficient enough to produce satisfactory yields.

Table 2. Comparison of IR Data of 5c.1, 5c.2, and 5c.3 from Literature Values

Product Entry	Experimental IR Data	IR Data Literature Values (cm^{-1}) (Weires *et al.*,2012)
5c.1	3052.54, 2746.33, 1603.33, 1432.15 – 1497.04, 1226.77 – 1275.88, 966.70, 834.15, 742.76	3053, 2746, 1682, 1448, 1274, 964, 831, 764
5c.2	3052.54, 2746.33, 1603.33, 1432.15 – 1497.04, 1226.77 – 1275.88, 966.70, 834.15, 742.76	
5c.3	3052.54, 2746.33, 1603.33, 1432.15 – 1497.04, 1226.77 – 1275.88, 966.70, 834.15, 742.76	

3.2 Synthesis of Benzimidazole Derivatives

$Fe_2(SO_4)_3/SiO_2$ as catalyst was confirmed to successfully facilitate the condensation reaction of (1) and 4c resulting to formation of benzimidazoles. The optimization data suggests that a 40% w/w of $Fe_2(SO_4)_3/SiO_2$ was able to elicit satisfactory yields after reaction and purification. 40% w/w composition of $Fe_2(SO_4)_3/SiO_2$ was then used as the optimum %w/w composition of the catalyst for the synthesis of aryl-benzimidazole analogues. Synthesized compounds were subjected to characterization and elucidation after synthesis by determining their melting point, IR-spectral data (using ATR), NMR (^1H and ^{13}C) and mass spectrometry (MS) analysis. Melting point for each synthesized product were determined and compared to literature values, whenever available (Tables 3 and 4). Moreover, data reveals that the target compounds have high melting points which range from 133 to 326 °C. Thorough literature search for the synthesized compounds 6b-6e, and 7a-7e was employed. However, no pieces of literature for these compounds, indicating that they have a high possibility of being a novel compound, were found.

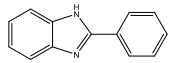
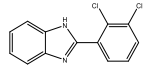
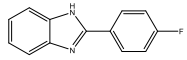
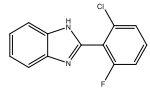
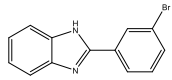
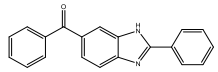
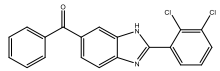
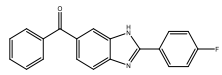
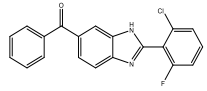
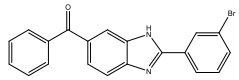
3.3 Characterization and Structure Elucidation of Synthesized Aryl-Benzimidazoles

In order to prove that the synthesized compounds were indeed benzimidazole analogues, samples were sent to the NMR Facility of the Institute of Chemistry, College of Science, University of the Philippines-Diliman, Quezon City, Philippines for NMR analysis (^1H, ^{13}C, and Correlation Spectroscopy). The structures of the different compounds were elucidated based on their ^1H and ^{13}C NMR spectra. Furthermore, samples were also subjected to FT-IR analysis for the identification confirmation of their functional groups and mass spectrometry analysis for the confirmation of their functional groups and molecular weights.

3.4 FT-IR Analysis

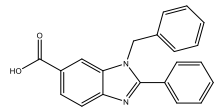
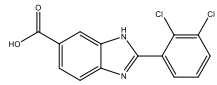
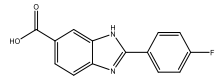
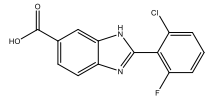
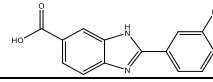
FT-IR analysis showed medium sharp peak around 1550-1600 cm^{-1} and a small sharp peak at 1220-1300 cm^{-1}. This indicates the presence of an N-H stretch and a C=N aromatic stretch, respectively, which corresponds to the aromatic imidazole functional group prominent in benzimidazole analogues. These are present in all synthesized compounds as shown in Figures 2, 3, and 4 for representative compounds 5c, 6c, and 7c, respectively.

Table 3. Melting point (Experimental and Literature) values of the synthesized 2-substituted-1H-benzimidazoles (5a – 5e, 6a – 6e)

Product Entry Code	Target Compound Molecular Structure	Melting Point Experimental (°C)	Melting Point Literature (°C)
5a		289-290	290-295 (Gogoi and Konwar, 2006) (Pouchert, 1983)
5b		237-238	224-226 (Naeimi and Alishahi, 2013)
5c		254-255	249-251) (Weires *et al.*,2012)
5d		216-217	ND
5e		247-248	ND (Dabhade *et al.*, 2009)
6a		185-186	**
6b		132-133	**
6c		242-243	**
6d		209-211	**
6e		220-221	**

**possible novel compound; ND-not determined by literature

Table 4. Melting point (experimental and literature) values of the synthesized Aryl-substituted-benzimidazoles (7a-7e)

Product Entry Code	Target Compound Molecular Structure	Melting Point Experimental (°C)	Melting Point Literature (°C)
7a		208-210	**
7b		322-323	**
7c		320-322	**
7d		324-326	**
7e		270-274	**

*Possible novel compound

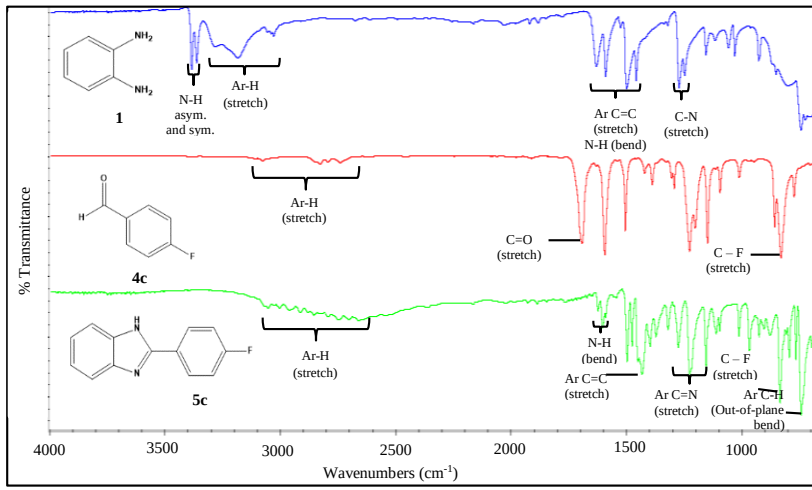


Figure 2. IR spectral comparison of o-phenylenediamine (1), 4-fluorobenzaldehyde (4c), and product 5c using FT-IR diamond ATR accessory (Silverstein *et al.*, 2005)

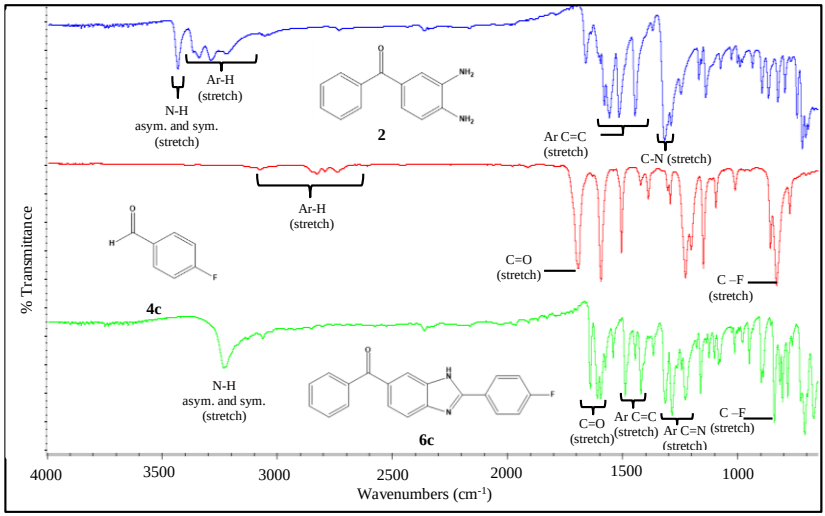


Figure 3. IR spectral comparison of (3, 4-diaminophenyl) (phenyl) methadone (2), 4-fluorobenzaldehyde (4c), and product 6c using FT-IR diamond ATR accessory (Silverstein *et al.*, 2005)

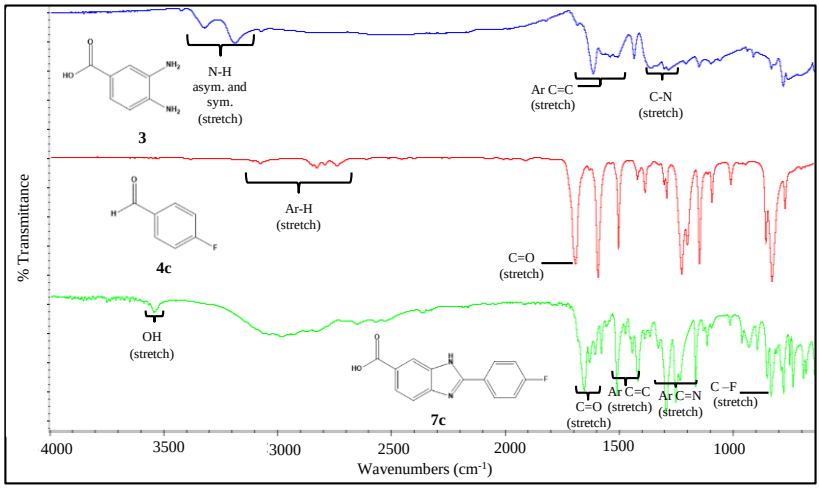
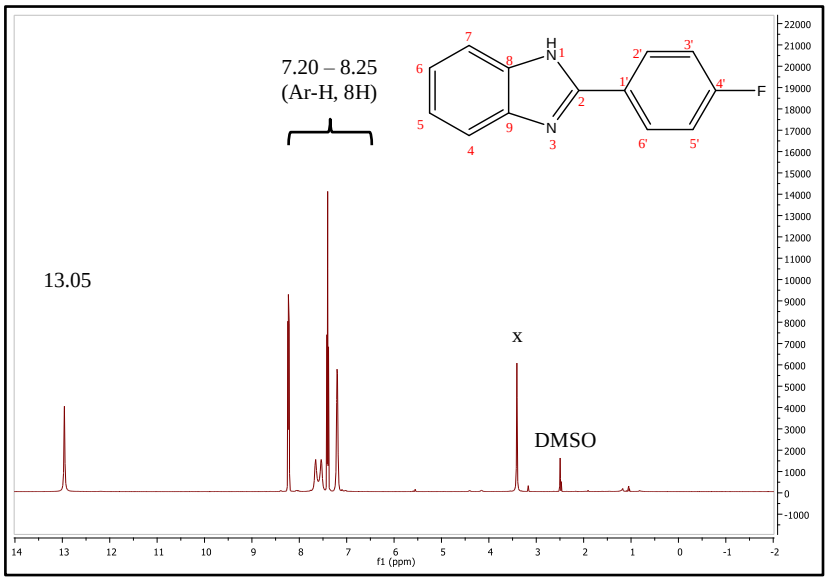


Figure 4. IR spectral comparison of 3,4-diaminobenzoic acid (3), 4-fluorobenzaldehyde (4c), and product 7c using FT-IR diamond ATR accessory (Silverstein *et al.*, 2005)

3.5 NMR and MS Analysis

For compounds 6a-6e, an intense sharp peak around 1600-1700 cm^{-1} describes a carbonyl group (C=O) suggesting the conservation of the (phenyl)methanone backbone as shown in the IR spectrum of 6c (Figure 3). While for compounds 7a-7e, sharp peaks around 1600-1700 cm^{-1} and a broad peak around 3,300-3,400 cm^{-1} describe the preservation of the carbonyl group of the carboxylic acid (COOH) substituent as shown in the IR spectrum of 7c (Figure 4). These results indicate the successful formation of an imidazole ring (Silverstein *et al.*, 2005).

^{1}H and ^{13}C NMR experimental data support the IR data of each synthesized compound and further confirm that compounds 5a-5e, 6a-6e, and 7b-7e were 2-substituted-1*H*-benzimidazoles. ^{1}H NMR peaks around 12.00-13.00 ppm connotes hydrogen attached to nitrogen (N-H peak). Proton peaks around 7.00-8.00 ppm indicates aromatic hydrogen. ^{13}C NMR peaks at around 150.00-160.00 ppm suggests the presence of a tertiary carbon existing in the imidazole core. ^{13}C NMR peaks at 190.00-200.00 ppm and 165.00-170.00 ppm are indicative peaks of a carbonyl carbon of the (phenyl) methanone group of compounds 6a-6e and the carboxylic acid group of compounds 7a-7e. Compound 7a was elucidated and was proven to be a 1,2-disubstituted benzimidazole supported by its ^{1}H and ^{13}C NMR spectra. Proton peaks around 5.5-6.0 ppm indicates a $-CH_2-$ carbon directly attached to nitrogen of the imidazole core. Presence of one singlet peak around 12.50-13.00 ppm assigned for the O-H group of carboxylic acid, and integration of the number of hydrogen atoms, at 16 hydrogen atoms, of the ^{1}H NMR spectrum support the total number of hydrogen atom for a 1,2-disubstituted benzimidazole. Carbon NMR peaks at 168.22 ppm for the carbonyl carbon (C=O) peak, 156.44 ppm for the tertiary carbon of the imidazole core, and 47.80 ppm for the $-CH_2-$ directly attached to the imidazole core further suggesting that compound 7a was a 1,2-disubstituted benzimidazole, specifically 2-phenyl-1H-benzo[d]imidazole-6-carboxylic acid. Figures 5 to 12 show the ^{1}H and ^{13}C NMR spectra of compounds 5c, 6c, 7c, and 7a, respectively.



x – impurities

Figure 5. ^{1}H NMR spectrum of 5c in DMSO-d6 at 500 MHz

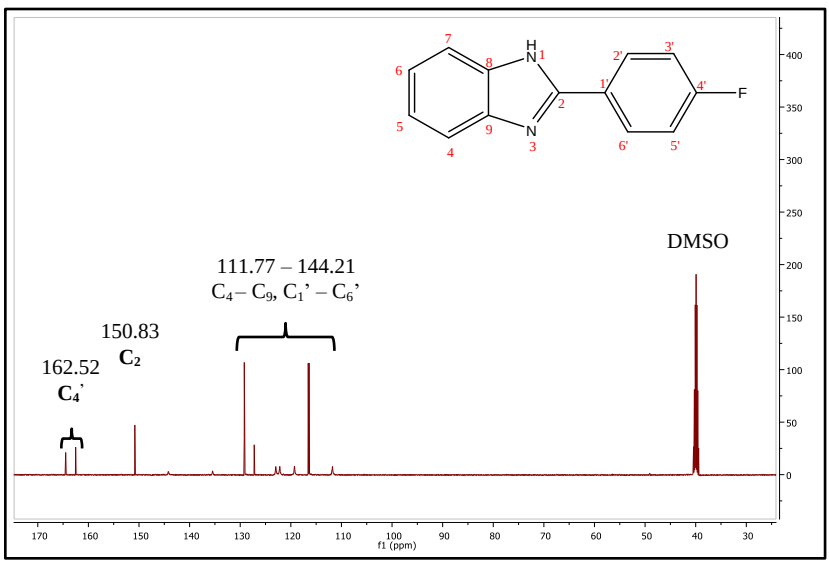
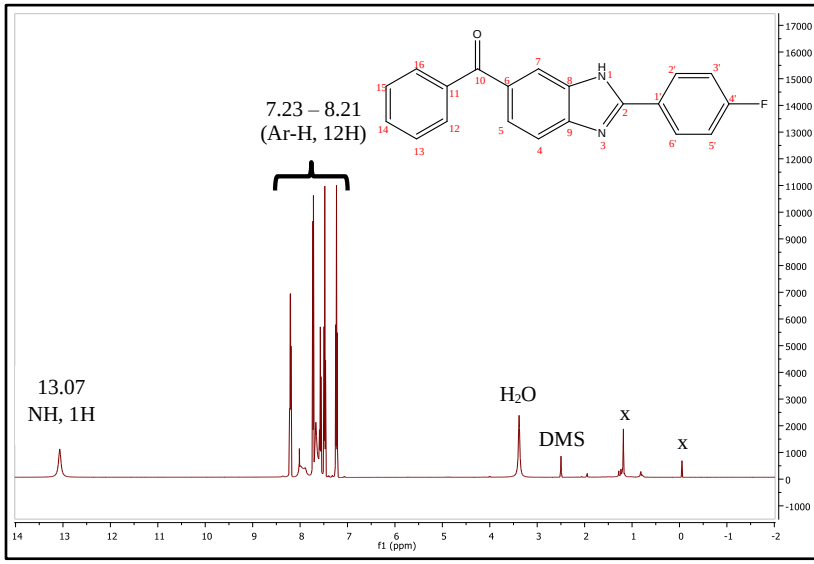


Figure 6. ^{13}C NMR spectrum of 5c in DMSO-d6 at 500 MHz



x – impurities

Figure 7. ^{1}H NMR spectrum of 6c in DMSO-d6 and $CDCl_3$ (1:1) at 500 MHz

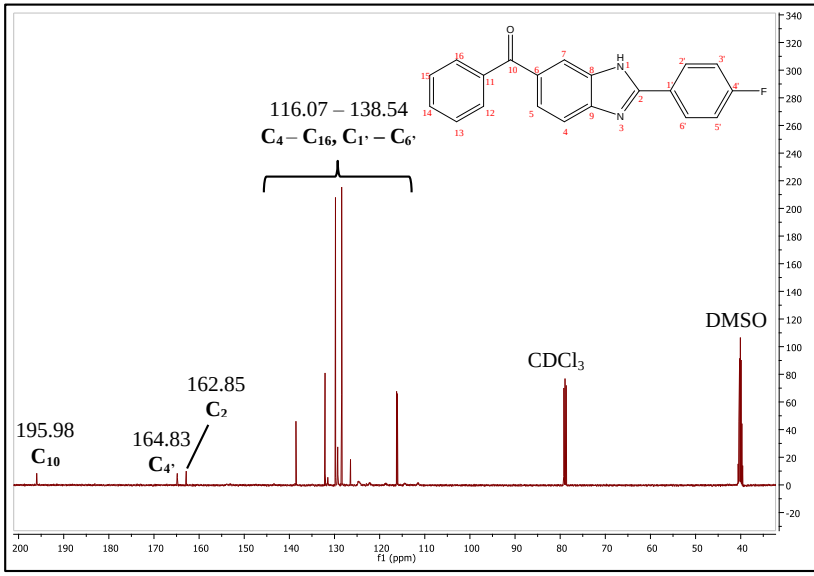
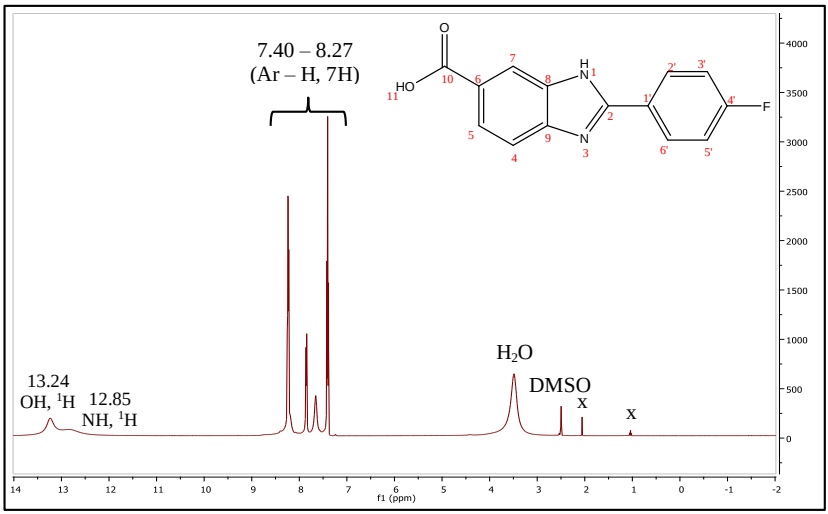


Figure 8. ^{13}C NMR spectrum of 6c in DMSO-d6 and CDCl3 (1:1) at 500 MHz



x - impurities

Figure 9. ^{1}H NMR spectrum of 7c in DMSO-d6 at 500 MHz

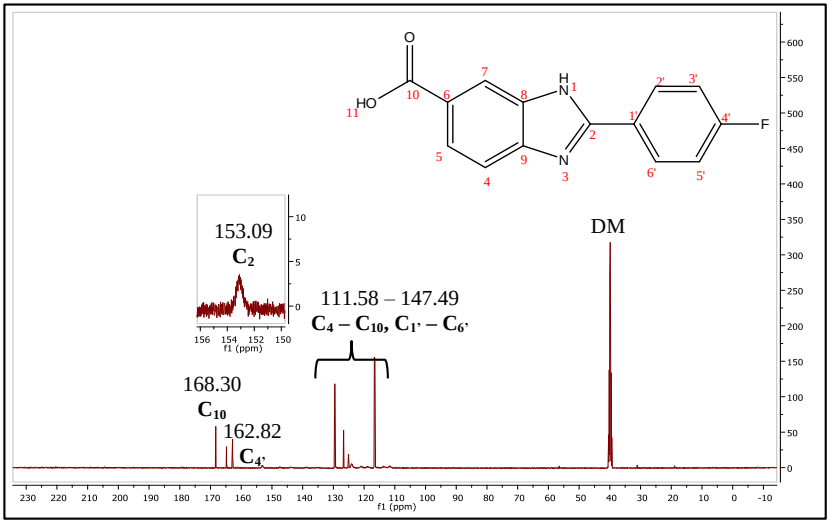


Figure 10. ^{13}C NMR spectrum of 7c in DMSO-d6 at 500 MHz

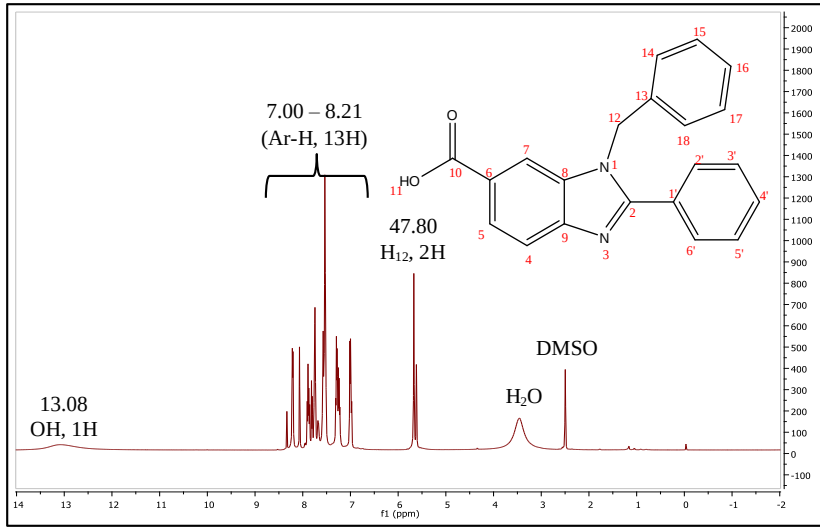


Figure 11. ^{1}H NMR spectrum of 7a in DMSO-d6 at 500 MHz

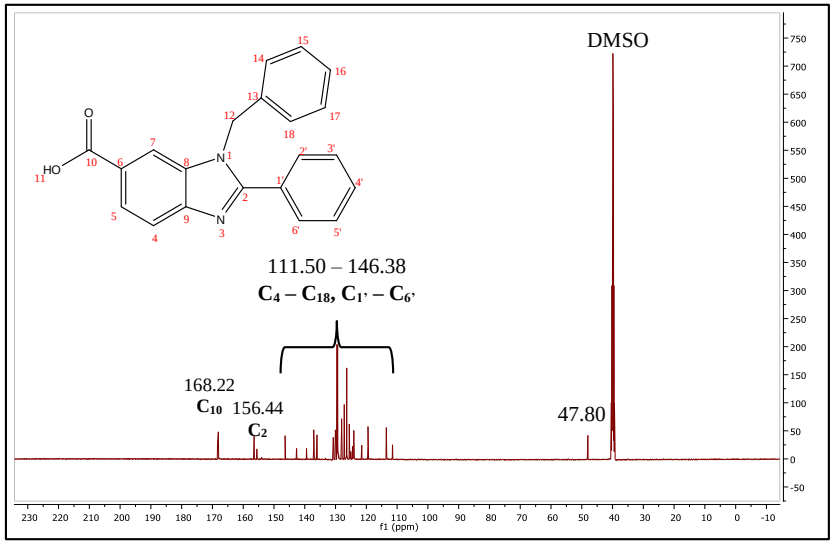


Figure 12. ^{13}C NMR spectrum of 7a in DMSO-d6 at 500 MHz

Moreover, the Structure of the synthesized compounds was further verified through ESI-Mass Spectrometry analysis. MS analysis showed the desired molecular weights of the target compounds as elucidated by the IR and NMR spectral data. Table 5 shows the theoretical molecular ion peaks (m/z) and experimental molecular ion peaks (m/z+1) of each compound together with their % yields and experimental melting point (°C). Additionally, the method gave satisfactory yields of about 22.62% - 51.94% together with a simple and reproducible method for the synthesis and purification of the synthesized products. Compound profile (Molecular Formula, Melting Point (°C), IR Data (cm^{-1}), ^{1}H and ^{13}C NMR Data, and Mass Spectral Data (m/z+1) for all the synthesized compounds are cited in the references section of this paper.

Table 5. Molecular weights, experimental base peaks, percent yield, and melting point of synthesized compounds

Product	R_1	R_2	Theoretical Molecular Ion (m/z)	Experimental Molecular Ion (m/z+1)	Yield (%)	Melting Point (°C)
5a	H	C_6H_5	194.08	195.0	38.10	289-290
5b	H	$C_6H_3Cl_2$	265.01	262.9	48.69	237-238
5c	H	C_6H_4F	212.07	213.0	45.75	254-255
5d	H	C_6H_3ClF	246.07	247.0	51.94	216-217
5e	H	C_6H_4Br	271.99	272.9	51.67	247-248
6a	C_7H_5O	C_6H_5	298.11	299.1	41.90	185-186
6b	C_7H_5O	$C_6H_3Cl_2$	366.03	367.0	22.62	132-133
6c	C_7H_5O	C_6H_4F	316.10	317.1	34.81	242-243
6d	C_7H_5O	C_6H_3ClF	350.06	351.0	41.66	209-211
6e	C_7H_5O	C_6H_4Br	376.02	379.0	40.86	220-221
7a*	COOH	C_6H_5	328.12	329.0	46.17*	208-210
7b	COOH	$C_6H_3Cl_2$	306.00	307.0	42.35	322-323
7c	COOH	C_6H_4F	256.06	257.1	38.15	320-322
7d	COOH	C_6H_3ClF	290.03	291.0	27.45	324-326
7e	COOH	C_6H_4Br	315.98	316.9	41.97	270-274

*disubstituted-benzimidazole derivative

3.6 Toxicity Assay

Synthesized compounds (5a-5e, 6a-6e, and 7a-7e) were subjected to brine shrimp lethality assay for preliminary assessment of their biological activities. Each compound was exposed to *A. salina* for 6 and 24 h for the acute and chronic toxicity data of each synthesized compounds. Analysis of obtained data from toxicity was subjected to the Reed-Muench method for calculating median lethal concentration (LC_{50}). Figure 13 displays compound 5a statistical calculation for LC_{50} determination (6 h) by obtaining the intersection of the graphs of live and dead test subjects, *A. salina*.

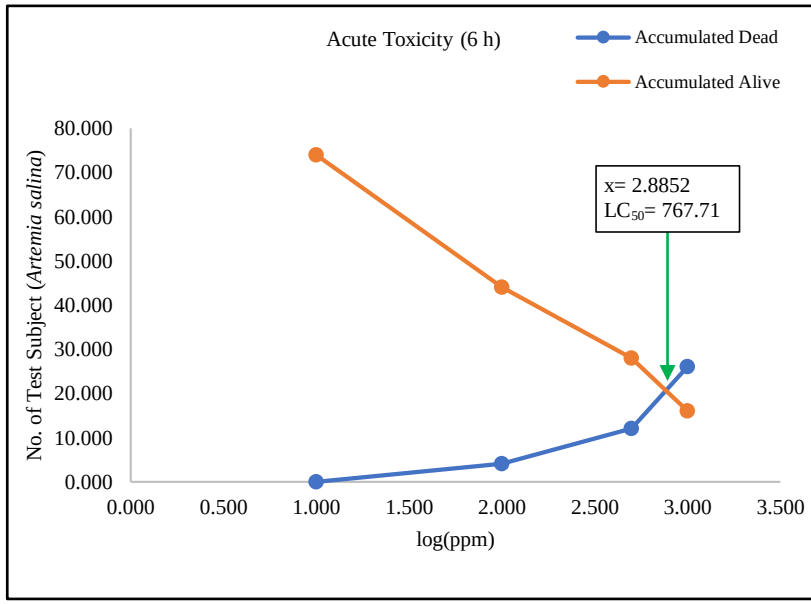
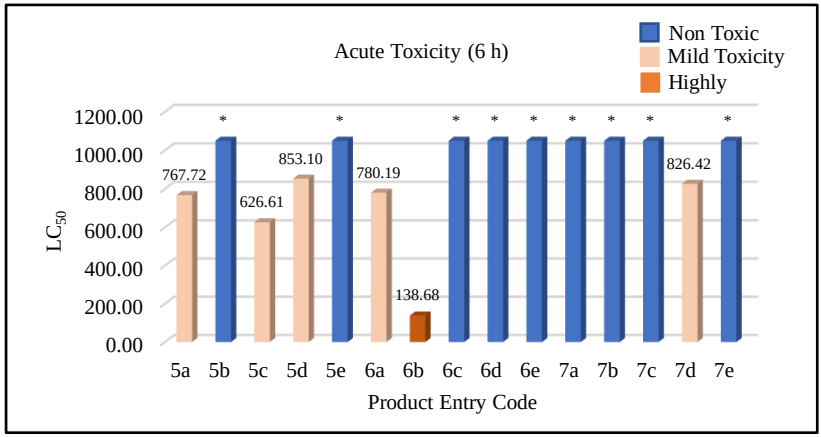


Figure 13. Compound 5a LC_{50} determination against brine shrimp *A. salina* using Reed-Muench method

Figure 14 and Figure 15 show the bar graphs for LC_{50} at 6 (acute) and 24 h (chronic) toxicities for all synthesized compounds. Based on Figure 14, compounds 5a, 5c, 5d, 6a, and 7d (light red) showed LC_{50} around 767.72, 626.21, 853.10, 780.19, and 826.42 ppm, respectively, at 6 h exposure to brine shrimp *A. salina* – suggestive of their mild toxicity. Compound 6b showed lowest value of LC_{50} at 138.68 ppm, indicating high toxicity at small concentrations. Other compounds (blue) have LC_{50} greater than 1000 ppm, signifying no toxicity effect at 6 h exposure to *A. salina*.

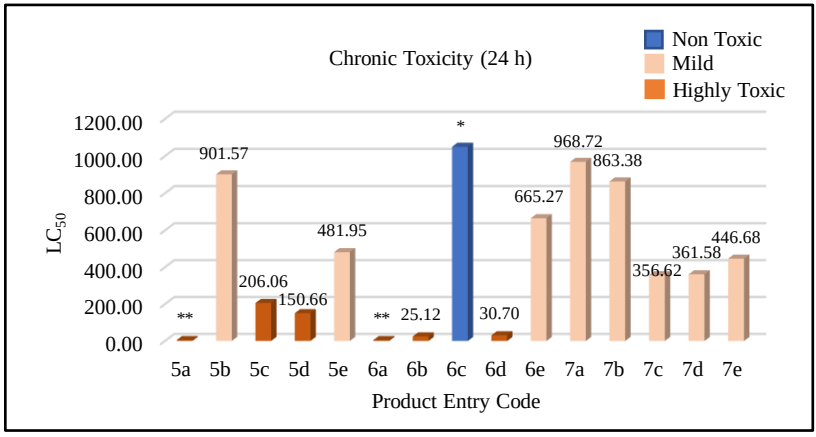
On the other hand, Figure 15 shows varying levels of toxicity from different compounds with compounds 5a and 6a bring about significantly low LC_{50} levels (<10 ppm) – implying very high toxicity levels at 50% mortality of the test subjects, *A. salina*, at 24 h exposure. Moreover, compounds 5c, 5d, 6b and 6d, also showed low levels of LC_{50} at 206.06, 150.66, 25.12, and 30.70 ppm, respectively, signifying high toxicity. Compounds 5e, 6e, 7c, 7d, and 7e showed satisfactory values of LC_{50} (481.95, 665.27, 356.62, 361.58, and 446.68 ppm), suggesting mild toxicity of the synthesized compounds at 24 h exposure to test subjects, *A. salina*. Compounds 5b, 7a, and 7b showed low toxicity levels with LC_{50} values at 901.57, 968.72, and 863.38 ppm,

respectively. Compound 6c offers no toxicity effect in either 6 or 24 h exposure to test subjects with LC_{50} greater than 1000 ppm for both conditions. These results denote that these compounds may exhibit possible pharmacological importance especially at chronic levels. Moreover, compounds 5a, 5c, 5d, 6a, 6b, and 6d must be studied further due to their high toxicity effect.



*LC_{50} greater than 1000 ppm

Figure 14. LC_{50} of synthesized compounds at 6 h exposure to *A. salina*



*LC_{50} greater than 1000 ppm; **LC_{50} less than 10 ppm

Figure 15. LC_{50} of synthesized compounds at 24 h exposure to *A. salina*

4. Conclusion

It can be concluded that a modified, simple, ecofriendly, and reproducible approach in the synthesis and purification of benzimidazole analogs can be carried out using $Fe_2(SO_4)_3/SiO_2$ as catalyst under room temperature conditions. Furthermore, the toxicity of the synthesized compounds varies from mild to high toxicity against *A. salina* indicating potential bioactivity of synthesized compounds particularly compounds 5a, 5c, 5d, 6a, 6b, and 6e. Moreover, this method endorses a reproducible approach in the synthesis of privileged compounds, which can be of paramount importance not only in the pharmaceutical industry but also in other fields of sciences.

5. Acknowledgement

The authors would like to acknowledge the Department of Science and Technology – Philippine Council for Health Research and Development (DOST-PCHRD) through the Drug Discovery and Development program for the financial support of this study, and the provision of important instruments such as the FT-IR (ATR) and NMR in the elucidation process of the synthesized compounds.

6. References

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