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# Synthesis and Analgesic Evaluation of Two Benzimidazole Derivatives

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**ABSTRACT**

Pain is associated with potential tissue damage, and Nonsteroidal anti-inflammatory drugs (NSAIDs) have proven to be effective in the management of pain and other conditions. Cyclooxygenase (COX) is the main target of NSAIDs. However, the use of NSAIDs come with various side effects such as gastric ulceration and bleeding, renal and cardiovascular complications, and generation of oxidative stress. This research synthesized benzimidazole derivatives to eliminate gastrointestinal toxicity. These compounds were synthesized through a condensation reaction with o-phenylene diamine and substituted aldehyde in the presence of a boric acid catalyst. The FT-IR, $^{1}$H-NMR, $^{13}$C-NMR spectra confirmed the structure of these compounds. Their analgesic activity was achieved through the acetic acid induced writhing test. The writhing responses were induced by intraperitoneal injection of 0.6% acetic acid solution in a volume of 1 ml/kg of body weight. The number of writhes was recorded withing 10 min and was compared to the control group. The synthesized compound 2-(benzo[d][1,3]dioxol-5-yl)-1H-benzo[d]imidazole (BP) was effective at 40 and 80 mg/kg while 2-(4-methoxyphenyl)-1H-benzo[d]imidazole (BM) was effective at 40 mg/kg only. The results of the biological evaluation suggested that BP displayed better analgesic activity than BM. Due to the absence of carboxylic acid function, these might also indicate safer interaction with the amino acids at the active site.

**Keywords:** Gastric Toxicity, Pain, Benzimidazole, NSAIDs

## INTRODUCTION

The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [1]. NSAIDs are the most widely used over-the-counter drugs as well as the most prescribed class of drugs for a

variety of conditions including pains, rheumatoid arthritis, osteoarthritis, musculoskeletal disorders, and other comorbid conditions [2].

Cyclooxygenase enzymes (COX-1 and COX-2) are of particular interest because they are the major target of NSAIDs, which bind to the COX active site preventing arachidonic acid (AA) reach to the catalytic pocket and hence, prostaglandins (PG) biosynthesis [3-5]. One of the mechanisms that has been associated with the adverse effects of NSAIDs is the generation of oxidative stress [2, 6]. NSAIDs are divided into two major groups: cyclooxygenase (COX) -2-selective inhibitors (COXIBs) and non-selective NSAIDs [7]. Inhibition of COX-1 reduces PG level in gastro intestinal track and results in gastric ulceration and bleeding. This is due to the interaction of the acidic group (COOH) of the non-selective NSAIDs with TYR355 and ARG120 at the enzyme active site [8, 9, 10]. COXIBs were introduced to eliminate gastric ulceration and retain analgesic and anti-inflammatory potential. COXIBs were withdrawn from the market due to cardiovascular complications [11, 12]. A literature search on the structure-activity relationship [13] of analgesics was conducted as a guide in synthesizing non-selective NSAIDs that are devoid of acidic groups to prevent interactions that cause gastric toxicity and have analgesic activity.

Benzimidazole scaffold (Figure 1), is an important biologically active heterocyclic compound that serves as one of the top ten most frequently employed five-membered nitrogen heterocyclic compounds among the US Food and Drug Administration (FDA) approved drugs [14]. The electron-rich nitrogen heterocycle of benzimidazole could readily accept or donate protons and easily allow the formation of diverse weak interactions, offering an advantage for it to bind with a broad spectrum of therapeutic targets, thereby exhibiting wide-ranging pharmacological activities [15]. Benzimidazoles have a structure that resembles naturally occurring purine nucleotides, which allows them to easily contact the biopolymers within the living system [16].

Literature searches revealed that these compounds have not been subjected to any target or tested for any biological activity. The aim is to synthesize compounds that can interact safely with the essential amino acids at the COX active site. These can be achieved with compounds that are devoid of acidic group in their structure. In contrast to non-selective NSAIDs, which consist of acidic group in their basic structure responsible toxicity.

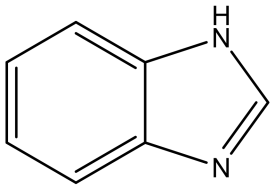
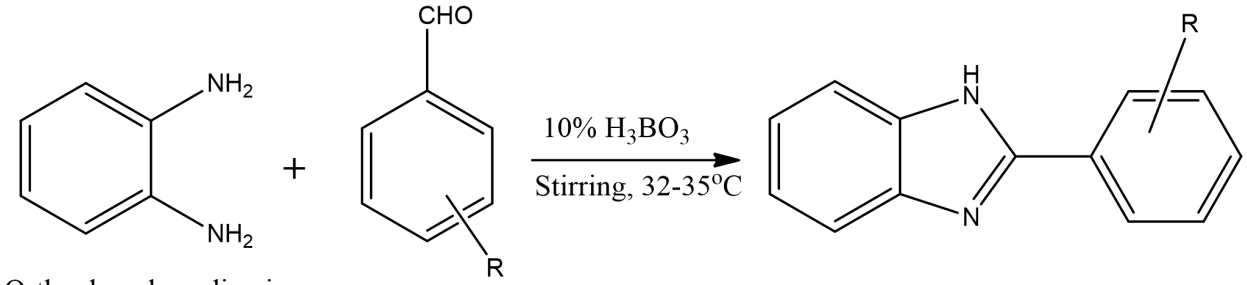



Figure 1: Benzimidazole Scaffold

## MATERIALS AND METHODS

The reagents and chemicals used were of laboratory grade and obtained from Sigma Aldrich. Nuclear Magnetic Resonance (NMR) (400 MHz Agilent NMR spectrometer, United Kingdom) was performed. The melting points of the two synthesized compounds were determined by the open capillary tube method and are uncorrected. The completion of the reaction was monitored by Thin Layer Chromatography (TLC) on pre-coated silica gel (HF254-200 mesh) aluminum plates from E-merk using ethyl acetate: n-hexane (5:1) as mobile phase. Spots were detected under the UV chamber. The IR spectra of the compounds were recorded with an Agilent FT-IR spectrophotometer. The 1H-NMR spectra of the synthesized compounds were recorded at 400 MHz NMR spectrophotometer in TMS. The test animals were obtained under the norms from the animal house in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

### Synthesis




Orthophenylene diamine

Substituted aldehyde

Scheme 1: Synthesis of Benzimidazoles

### Procedure

Orthophenylene diamine (0.01 mol) was condensed with two substituted aldehydes (0.01 mol) in 20 ml 10% boric acid solution as shown in scheme 1. The mixture was stirred for1h 30-35 $^0$C. The solution was filtered, washed with distilled water and recrystallized with ethanol [17].

## Pharmacological Evaluation

### *Acute oral toxicity studies*

In the present study, the acute oral toxicity of the synthesized compounds was performed by the acute toxic class method (OECD guideline 425). Animals were observed individually after dosing at least once during the first 30 min; periodically during the first 24 h with special attention given during the first 4 h and daily thereafter, for a total of 14 days. No mortality was observed with the administered dose of 200 mg/kg.

### *Analgesic Activity*

Analgesic activity was determined by using a 0.6% acetic acid solution (abdominal constriction) assay. The mice were weighed and divided into three groups, control, reference, and experimental groups. Six mice were in each group and were dosed at 20, 40, and 80 mg/kg. In this assay, at the time interval of 60 min after the administration of the dosing vehicle (control), reference standard (ibuprofen), and test sample (BM and BP), the writhing responses were induced by intraperitoneal injection of 0.6% acetic acid solution in a volume of 1 ml/kg of body weight. The number of writhes (constriction of the abdomen, turning of trunk, and extension of hind limbs) for each mouse was recorded. The writhing response occurred within 60 seconds and the number of writhes was recorded within 10 minutes. The number of writhes in each group was compared to that of the control group [18,19].

## RESULTS AND DISCUSSION

2-(4-methoxyphenyl)-1H-benzo[d]imidazole; BM-$C_{14}H_{12}N_2O$; Solid: yellow; yield: 64%; (mp 101–104$^o$C). FT-IR: 3478 (N-H stretching), 3373 (C=C aromatic stretching), 1595 (N=C stretching), 1244 (C-O-C stretching) $cm^{-1}$. $^1$H NMR ($CDCl_3$): δ 8.48 (s,1H-N), 7.02 (m, $J$ = 5.9 Hz, 2H, 6&9), 6.76 (m, $J$ = 15.4 Hz, 2H, 7&8), 7.89 (d, $J$ = 9.1 Hz, 2H, 3'&5'), 8.02 (d, $J$ = 2.8 Hz, 2H, 2'&6'), 3.90 (s, 3H, 7'). $^{13}$C NMR (400 MHz, TMS): δ 55.02 (7'), 114.15 (8), 114.51 (7), 115.52 (9), 117.98 (6), 118.32 (1'), 127.45 (6'), 128.38 (2'),129.70 (5'), 130.82 (3'), 137.20 (5), 142.53 (4), 157.04 (4'), 161.87 (2).

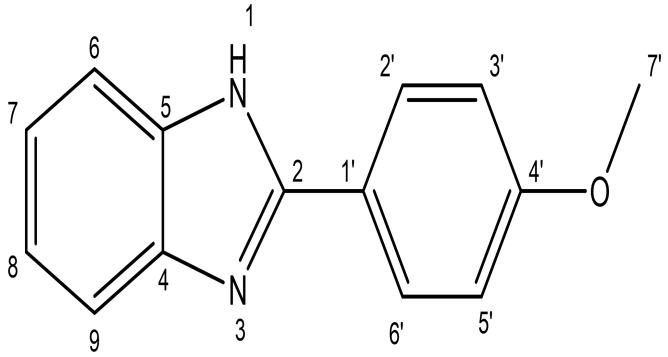



Figure 2: 2-(4-methoxyphenyl)-1H-benzo[d]imidazole (BM)

2-(benzo[d][1,3]dioxol-5-yl)-1H-benzo[d]imidazole; BP-$C_{14}H_{10}N_2O_2$, Solid: White, yield: 71% (mp 102–105°C). FT-IR: 3436 (N-H stretching), 3343 (C=C aromatic stretching), 1599 (N=C stretching), 1044 (O-C-O stretching) $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ 8.44 (s,1H-N), 6.78 (d, *J* = 24.1 Hz, 2H, 5'&6'), 7.07 (m, *J* = 15.5, 8.4 Hz,2H, 6&9), 7.58 (s, 1H, 2'), 6.86 (m, *J* = 41.8 Hz, 2H, 7&8), 6.07 (s, 2H, 7'). $^{13}C$ NMR (400 MHz, TMS): δ 101.59 (7'), 106.50 (7), 107.86 (8), 115.06 (9), 117.08 (6), 118.43 (6'), 125.38 (5'), 127.20 (2'), 131.70 (1'), 137.82 (5), 142.04 (4), 148.98 (4'), 150.37 (3'), 156.87 (2).

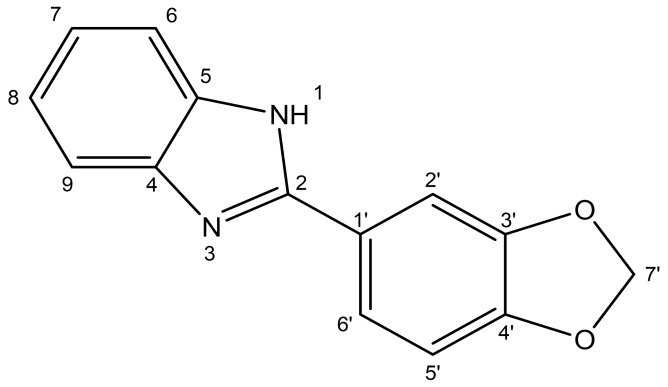



Figure 3: 2-(benzo[d][1,3]dioxol-5-yl)-1H-benzo[d]imidazole (BP)

**Analgesic Activity**

The biological activity of these compounds has been evaluated for the first time. The analgesic activity of the compounds BM and BP was observed at doses of 20, 40, and 80 mg/kg at interval of 60 minutes. The standard drug, ibuprofen inhibits the writhing responses whereas the compounds exhibit analgesic effects at 40 and 80 mg/kg for BP and 40 mg/kg for compound BM. The results indicated that compound BP showed analgesic activity comparable to Ibuprofen.

Table 1. Analgesic activity of the synthesized compound (BM)

| Compounds | Dose (mg/kg) | MEAN±SEM (NW) 60 min |
|---|---|---|
| Control | - | 29.00±9.678 |
| BM | 20 | 19.00±11.038 |
| BM | 40 | 1.50±1.500[a] |
| BM | 80 | 9.00±3.674 |
| IBP | 10 | 0.25±0.250[a] |

Number of writhing responses (NW) at 60 min.
Distilled water (DW)
Ibuprofen (IBP)
Data are express as mean ± SEM. The statistical difference was determined by one-way ANOVA, followed by post-hoc Dunnett's test.
Mean values having letter (a: between groups) are significantly different ($P<0.05$)

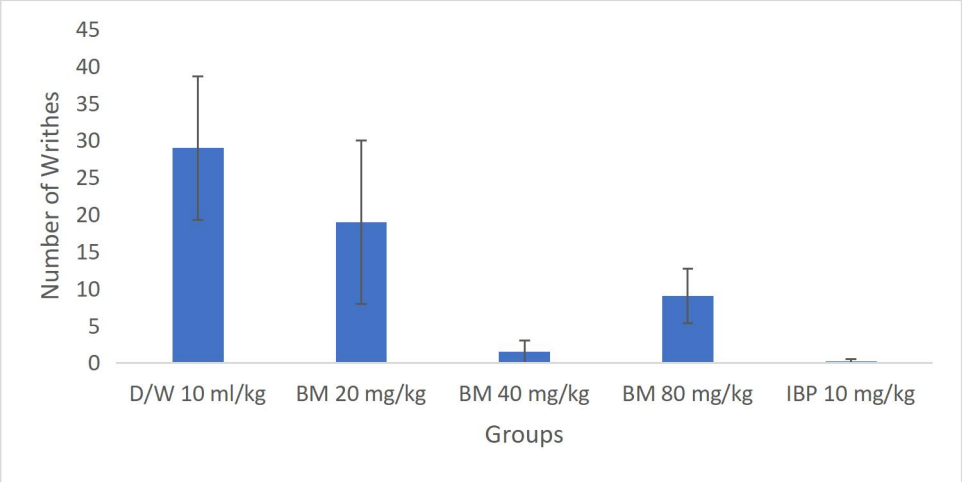



Figure 4: Graph displaying the analgesic effect of BM

Table 2. Analgesic activity of the synthesized compound (BP)

| Compounds | Dose (mg/kg) | MEAN±SEM (NW) 60 min |
|---|---|---|
| Control | - | 29.00±9.678 |
| BP | 20 | 6.75±6.750 |
| BP | 40 | 0.25±0.250[a] |
| BP | 80 | 0.25±0.250[a] |
| IBP | 10 | 0.25±0.250[a] |

Number of writhing responses (NW) at 60 min.
Distilled water (DW)
Ibuprofen (IBP)
Data are express as mean ± SEM. The statistical difference was determined by one-way ANOVA, followed by post-hoc Dunnett's test.
Mean values having letter (a: between groups) are significantly different ($P<0.05$)

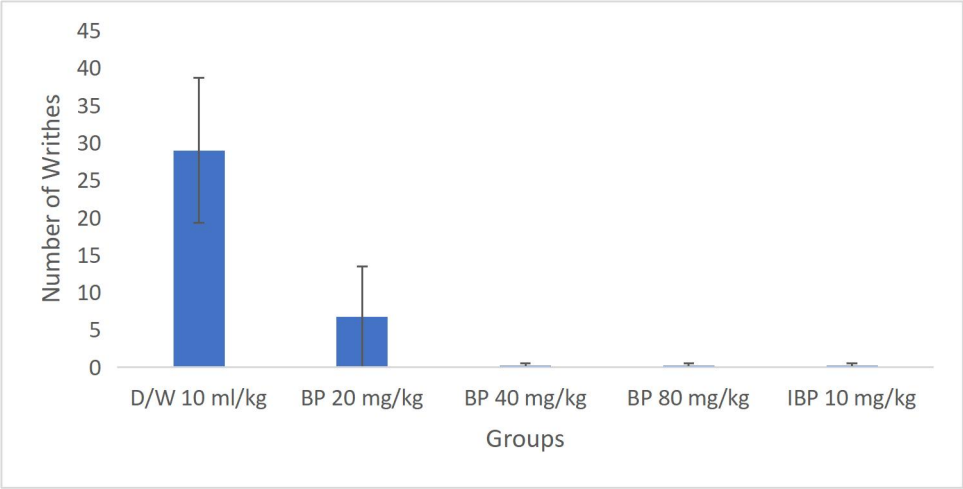



Figure 5: Graph displaying the analgesic effect of BP

## CONCLUSION

In this research two compounds that are devoid of acidic group were successfully synthesized. The results of biological evaluation suggested that BP displayed better analgesic activity than BM and due to the absence of carboxylic acid function these might also indicate safer interactions with the amino acids at the active site, indicating that these compounds are much safer on comparison with ibuprofen. The study can be extended by performing molecular docking, molecular dynamic simulation and experimental pharmacokinetic study to evaluate the safety and therapeutic efficacy of these compounds.

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