Synthesis, Characterization and Biological Evaluation of Some Novel Benzimidazole Derivatives

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***Abstract*—**The synthesis of a series of propynamide benzimidazolines derivatives by Michael addition of amino benzimidazoles on allenic acids in ethanol under reflux conditions is reported. The compounds were isolated in good yields and fully characterized by spectral and microanalytical data. Excellent antibacterial activity was recorded with values ranging from 62.5 µg/mL to 500 µg/mL.

***Keywords*—**Benzimidazole, Michael addition, biological activity

I. Introduction

The therapeutic journey of benzimidazoles started in 1944 when Woolley speculated that they simulate purines biological behavior [1]. Over the years of systematic research, the benzimidazole unit has emerged as an important heterocyclic unit because of its widespread occurrence in bioactive compounds like antiparasitic, antiulcer, antiviral and anti-inflammatory agents [2]–[4]. Benzimidazoles can be identified as a "Master Key" because they are part of the core structure of many biological active compounds such as vitamin-B12, etc. They have been reported in literature to possess antihypertensive [5]-[6], anti-inflammatory [7]–[9], anti-oxidant [10]-[11], antitumor [12]-[13], and antidiabetic [14] properties. The report of Iwahi and Satoh in the year 2000 on the antibacterial activities of 2-(substituted pyridyl methylsulfinyl)benzimidazole against *Campylobacter pylori* prompted many research activities on their antimicrobial potential [15]. This yielded several compounds whose activity was better than some commercially available antibiotics. Attachment of some other heterocycles to the benzimidazole nucleus resulted in compounds with interesting antifungal and antibacterial activities [16]–[20]. Some benzimidazoles derivatives have also been found to have excellent activities against, methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant *Staphylococcus epidermis* (MRSE) [21], [22]. With the rise of antimicrobial resistance worldwide, there is an urgent need to produce more effective therapeutic alternatives [23].

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II. General Procedures

A. Materials and Methods

Unless otherwise specified, the starting materials were commercially available. Solvents were purified by conventional methods before use. Infra red (IR) spectra were recorded using a Perkin Elmer (spectrum 100) FT-IR SPECTROMETER. ^{1}H NMR (300 MHz), and ^{13}C NMR (75 MHz) were measured on a Mercury-300 broadband "univn200" NMR spectrometer. Chemical shifts are reported in parts per million (ppm, δ), downfield from residual solvents peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t)…Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). The bacterial strains were obtained from Davies diagnostics.

B. Bacteria

Fourteen bacterial strains (Gram-positive bacteria: *Bacillus cereus* (ATCC10876), *B. subtilis* (ATCC19659), *Enterococcus faecalis* (ATCC13047), *Mycobacterium smegmatis* (MC^{2}155, kind gift from the Centre of Excellence in Biomedical TB Research, University of the Witwatersrand), *Staphylococcus epidermidis* (ATCC14990) and *S. aureus* (ATCC25923). Gram-negative bacteria: *Enterobacter cloacae* (ATCC13047), *Escherischia coli* (ATCC25922), *Klebsiella oxytoca* (ATCC8724), *K. pneumonia* (ATCC13882), *Proteus mirabilis* (ATCC7002), *Pseudomonas aeruginosa* (ATCC27853), *Enterobacter aerogenes(*ATCC13048*)* and *Proteus vulgaris(*ATCC6380*)* were cultured overnight in Mueller-Hinton broth at 25 °C; Merck Chemicals, SA). The turbidity of the culture solutions was adjusted to match a 0.5 McFarland standard within 15 minutes prior to antibacterial testing.

C. Antibacterial Testing

Minimum inhibitory concentrations (MIC) of all the strains were determined by the broth microdilution assay described in literature [24] . The test compounds were accurately weighed and dissolved in DMSO to yield 1000µg/ml. The dissolved compounds were then serially diluted in Mueller-Hinton broth till the lowest concentration of 15.62µg/ml. All dilutions were tested two-fold against each bacterial strain. 100µl of the

bacterial suspension was mixed with 100µl of pre-diluted test compound in a 96 microwell plate and left to incubate overnight at 37 °C. 10µl of a 0.02% (w/v) tetrazolium sodium solution was added to each well and the plates were re-incubated for 2 hours. Visual change of the solution from blue to pink indicated that the bacteria were still alive. MIC was determined as the minimum concentration of compound where no color change could be observed. The MIC of all strains tested were compared to two reference antibiotics (nalidixic acid and streptomycin sulphate). This was due to the fact that whilst streptomycin is a broad based antibiotic, nalidixic acid has been shown to be exclusively active against Gram-negative bacteria [25].

D. Synthesis of Synthesis of prop-2-ynamide-iminobenzimidazoles derivatives

Preparation of propynamide benzimidazolines derivatives: Phenyl or n-pentylpropyne acetylenic acids were heated under reflux with 2-aminobenzimidazoles or 5, 6-dimethyl-2-aminobenzimidazoles in ethanol overnight. A solid was recovered after solvent evaporation.

5a: *N-(1,3-dihydrobenzimidazol-2-ylidene)-3-phenyl-prop-2-ynamide*

Yield 80 %, amorphous powder, **^{1}H NMR (DMSO, 300 MHz)** δ (ppm) 7.11 (m, 1H); 7.28(m, 2H), 7.39(d, 2H, $^3J_{HH}$ =6.8); 7.47(m, 4H), 8.27(s, 2NH_2). **^{13}C NMR (DMSO, 75 MHz)** δ (ppm): 72.30; 83.00;116.96; 117.62; 122.93; 123.97; 124.33; 129.00; 129.32; 129.65; 143.26; 143.62; 143.97, 147.46; 154.77. **IR (film, cm^{-1})**: 3228; 2999; 2879, 2765, 2663;2017; 2883; 2772; 2202; 1736; 1684; 1613; 1557; 1360; 1272; 1208; 1033; 929; 889; 826; 749; 674. $[M+H]^+$: m/z 262.

5b: *N-(5,6-dimethyl-1,3-dihydrobenzimidazol-2-ylidene)-3-phenyl-prop-2-ynamide*

Yield 85 %, amorphous powder, **^{1}H NMR (DMSO, 300 MHz)** δ (ppm): 4.98(s, 2H), 5.72 (m, 3H), 5.80(m, 2H). **^{13}NMR (DMSO, 75 MHz)** δ (ppm): 15.08; 73.83; 84.58; 118.80; 119.12; 124.41; 125.20; 126.45; 127.81; 130.50; 131.16; 144.74; 145.10; 145.46; 148.83; 156.34. **IR (film, cm^{-1})**: 3456;2957; 246; 2200; 139; 1673; 1576; 1366; 1217; 1012; 960; 811; 742. $[M+H]^+$: m/z 290

5c: *N-(1,3-dihydrobenzimidazol-2-ylidene)oct-2-ynamide*

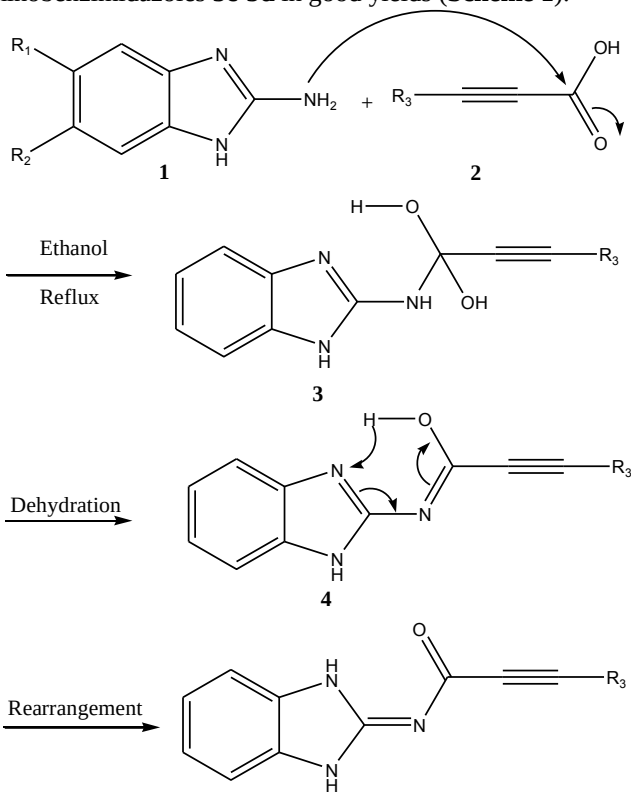
Yield 82 %, amorphous powder, **^{1}H NMR (DMSO, 300 MHz)** δ (ppm): 0.81(t, 2H, $^3J_{HH}$ =7.20); 1.15(m, 4H), 1.30(m, 2H); 2.21(t, 2H, $^3J_{HH}$ =1.9); 2.30(s, 2H) 715(m, 2H); 7.25(m, 2H); 7.39(m, 1H). **^{13}C NMR (DMSO, 75 MHz)** δ (ppm): 12.30; 16.00; 22.00; 25.69; 75.60; 85.95; 116.98; 117.31; 129.03; 129.69; 143.23; 143.59; 143.95; 148.00; 155.20. **IR (film, cm^{-1})**: 3458; 2970; 2948; 2238; 2208; 1740; 1680; 1609; 1555; 1488; 1441; 1365; 1216; 1093; 1025; 838; 775; 687. $[M+H]^+$: m/z 256

5d: N-(5,6-dimethyl-1,3-dihydrobenzimidazol-2-ylidene) oct-2-ynamide

Yield 88 %, amorphous powder, **^{1}H NMR (DMSO, 300 MHz)** δ (ppm): 0.859(t, 2H, $^3J_{HH}$ =6.3); 1.29 (m, 4H), 1.43 (m, 2H); 2.50(t, 2H, $^3J_{HH}$ =1.8); 7.04(s, 2H); 8.34(s, 2H). **^{13}C NMR (DMSO, 75 MHz)** δ (ppm): 14.50; 18.36; 20.30; 22.30; 28.17; 31.12; 80.22; 80.69; 112.38; 129.97; 130.88; 152.68; 159.57. **IR (film, cm^{-1})**: 3452; 3023; 2969; 2920; 2717; 2232; 1738; 1679; 1606; 1554; 1481; 1439; 1366; 1217; 1030; 845; 777; 689. $[M+H]^+$: m/z 284.

III. Results and Discussion

The conjugate addition of 2-aminobenzimidazole **1** to acetylenic acids **2** followed by a prototropic rearrangement yielded the corresponding (3-pentyl)-prop-2-ynamide-iminobenzimidazoles **5a-5b** and (3-phenyl)-prop-2-ynamide-iminobenzimidazoles **5c-5d** in good yields (**Scheme 1**).



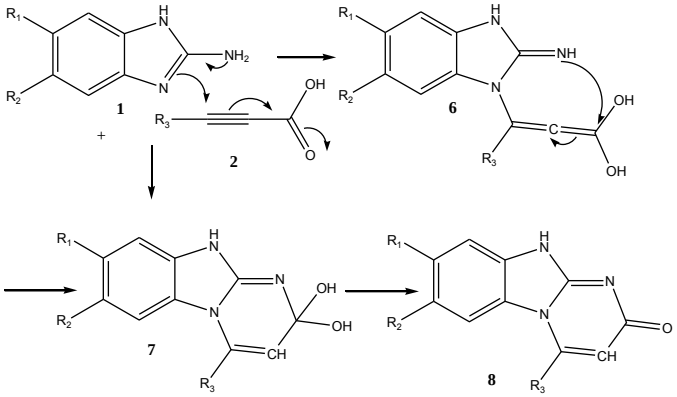
5a R_1 = R_2 = H, R_3 = Ph
5b R_1 = R_2 = CH_3, R_3 = Ph
5c R_1 = R_2 = H, R_3 = *n*-Pentyl
5d R_1 = R_2 = CH_3, R_3 = *n*-Pentyl

Scheme 1: Synthesis of prop-2-ynamide- iminobenzimidazoles deriva tives

The reaction of **2** and 2-aminobenzimidazole proceeds by the initial attack of the 2-amino group on the carbon C-3. The IR, ^{1}H-NMR, ^{13}C-NMR and the mass spectral data of the products are consistent with the structures assigned to **5a-5d**. The carbon-carbon triple bond stretch appears around 2200cm^{-1}. A strong carbonyl stretching is seen around 1736 cm^{-1} and the imine stretch just follows at 1680 cm^{-1}. In the proton NMR spectra of compounds **5**and **5b**, the aromatic protons appear at their expected position in the downfield region. For compounds **5c** and **5d**, the aliphatic protons appear in the upfield region. In the ^{13}C NMR spectrum of all the compounds, the carbonyl carbon and the imine carbon appear in the downfield region respectively around δ 159 and 152 ppm. The carbon triple bond signals are between δ 70 and 80 ppm.

The aromatic carbons appear in the downfield region as expected.

There is no indication of an initial attack from the internal amino group as suggested in **Scheme 2** as no pyrimido benzimidazoles ketones **8** were recovered.



Scheme 2: An alternative mechanistic pathway

All the synthesized compounds were tested against 14 strains of bacteria. **Table 1** summarizes the results for compounds **5a-5d** against the tested bacteria. All the compounds showed an activity against all the strains.

All the compounds showed better activity than Nalidixic acid against *Enterococcus faecalis, Escherichia coli, Mycobacterium smegmatis,Pseudomonas aeruginosa* and *Staphylococcus aureus*. We also recorded a MIC of 250 μg/ml against *Enterococcus cloacae* which is much better than that of Streptomycin.

The best activity was showed by compound **5b** against *Mycobacterium smegmatis*, which performed better than nalidixic acid with a MIC of 62.5 μg/ml.

TABLE I: MIC OF PROP-2-YNONE- IMINOBENZIMIDAZOLES DERIVATIVES ((MG/ML))

Bacterial strain	5a	5b	5c	5d	Streptomycin	Nalidixic acid
Bacillus cereus	500	500	250	250	32	32
Bacillus subtilis	125	250	250	125	16	16
Enterococcus faecalis	500	250	250	250	128	>512
Enterobacter cloacae	250	250	250	250	>512	16
Enterobacter aerogenes	500	500	500	250	16	256
Escherichia coli	500	250	250	250	64	>512
Klebsiella oxytoca	250	250	250	250	16	8
Klebsiella pneumoniae	500	125	500	250	64	64
Mycobacterium smegmatis	125	62.5	125	250	<4	>512
Pseudomonas aeruginosa	250	250	250	250	64	>512
Proteus mirabilis	500	500	500	500	128	32
Proteus vulgaris	500	250	250	500	32	8
Staphylococcus aureus	500	250	250	250	64	512
Staphylococcus epidermidis	250	250	500	>500	8	64

IV Conclusion

In conclusion, we have reported the synthesis of 4 novel heterocycles in good yields via the Michael addition of 2-aminobenzimidazoles on acetylenic acids. These compounds have been fully characterized by various spectroscopic methods. They have also been tested for biological activity and were tested against 14 strains of bacteria. *N-(5,6-dimethyl-1,3-dihydrobenzimidazol-2-ylidene)-3-phenyl -prop-2-ynamide* **5b**, showed very good activity against *Mycobacterium smegmatis* compared to Nalidixic acid.

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