# New Antibacterial Diazotized Derivatives of 5-Amino Azaheterocycles

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**Summary:** Six new diazotized derivatives of pharmacologically important azaheterocycles (2a-d, 4a-b) were prepared from their corresponding 5-amino benzimidazoles (1a-d) and benzimidazolones (3a-b). All new compounds have been characterized on the basis of their IR, NMR and Mass spectral data. Antibacterial activity of these compounds is also been reported.

Key words: azaheterocyle, benzimidazole, benzimidazolone, diazotization, antibacterial.

## Introduction

Nitrogen derivatives of heterocycles are versatile building blocks in several areas of advanced materials chemistry [1]. They also exhibit different pharmacological properties due to the possibility of oxidation of nitrogen or delocalization of electrons in molecule [2]. Diazotization is one of the familiar reactions to obtain nitrogen derivatives, and azo dyes of many heterocycles with variety of properties have previously been explored [3-6]. Benzimidazoles and benzimidazolones are important azaheterocyclic systems which exhibits a broad range of pharmacological activity [7-16]. Among nitrogen derivatives of benzimidazole, 2-amino derivatives are widely studied with array of biological activities [17-25] whereas 5-amino derivatives are rarely reported [26-29]. Furthermore, only few examples were found for azo dyes of these heterocylic derivatives [30-32]. In this perspective, we aimed to explore the diazotized derivatives of 5-amino benzimidazoles and benzimidazolones, in continuation of our previous report of their peptides analogs [33, 34].

## Results and Discussion

5-Amino benzimidazoles **1a-d** and benzimidazolones **3a-b** were synthesized by reported methods [33-37], involving conventional Phillips condensation [38], N-alkylation with alkyl halides and $K_2CO_3$ [36], nitration with $HNO_3/H_2SO_4$ at low temperature [39], followed by reduction of 5-nitro group to their corresponding amino analogs with Zn/HCl [33-34] (Fig. 1). These 5-Amino analogs of benzimidazoles **1a-d** and benzimidazolones **3a-b** were diazotized by using sodium nitrite and *β*-naphthol to obtain six new compounds **2a-d**, **4a-b** respectively as shown in Fig. 2 and 3.

IR spectrum of compounds **2a-d** revealed absorption at 1510, 1504, 1545, and 1507 $cm^{-1}$ for diazo (N=N) linkage and at 1698, 1685, 1695, and 1663 $cm^{-1}$ for C=N bond. Broad signals between 3425-3010 $cm^{-1}$ were observed for –OH of naphthol.

EIMS spectrum of all diazotized products **2a-d** exhibited more or less parallel fragmentation pattern. Molecular formulae of **2a-d** were confirmed from their molecular ion $M^{+\bullet}$ peaks observed at *m/z* = 316, 302, 316, 330 respectively. The presence of diazonium linkage was confirmed by ion peaks appeared at *m/z* = 172, 158, 172, and 186 correspondingly.

$^{13}C$ NMR of *5-(2′-hydroxynaphthyl diazo)-1,2-dimethyl benzimidazole* (**2a**), reveals the presence of nineteen carbons in the molecule, including seventeen aromatic and two methyl carbons. In $^{1}H$-NMR spectrum, nine protons were observed in aromatic region along with two methyl groups. H-4 is under inductive and resonance effect so it resonated at low field and appeared as a doublet at 8.57 ppm with coupling constant of 3.8 Hz for *meta* coupling. H-6, also resonated relatively downfield at 7.76 ppm as dd pattern with *J* = 6.6, 3.7 Hz revealing *ortho* and *meta* coupling. A doublet of one proton at 7.81 ppm with coupling constant of 6.9 Hz was assigned to H-3′. A multiplet corresponding to three protons at 7.59 ppm has been assigned to H-4′, H-6′, H-7′, while another doublet of triplet at 7.40 ppm is designated to H-5′. H-8′ appeared as a doublet at 7.16 ppm (*J* = 8.1, 3.4 Hz). The methyl group attached to the nitrogen appeared as a singlet at 3.80 ppm, whereas another methyl group attached to carbon resonated as a singlet, relatively upfield at 2.64 ppm.

$^{1}H$-NMR spectrum of compound **2b** exhibited a singlet of 3H at 3.81 ppm indicated N-$CH_3$ group. Nine aromatic protons were observed in the range of $\delta_H$ 7.14-8.34 ppm in the set of five different sets of splitting. H-4 resonated at 8.34 ppm

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with coupling constant of 3.5Hz for *meta* coupling. A doublet of 1H at 7.78 was designated to H-3, whereas H-8` is observed as doublet of doublet at 7.14 ppm. A dt pattern is observed for H-5` at 7.38 ppm. Other five aromatic protons appeared as multiple*t* between $\delta_H$ 7.55-7.71 ppm. $^{13}$C NMR of **2b** further confirmed the presence of one methyl and seventeen aromatic carbons in the compound.

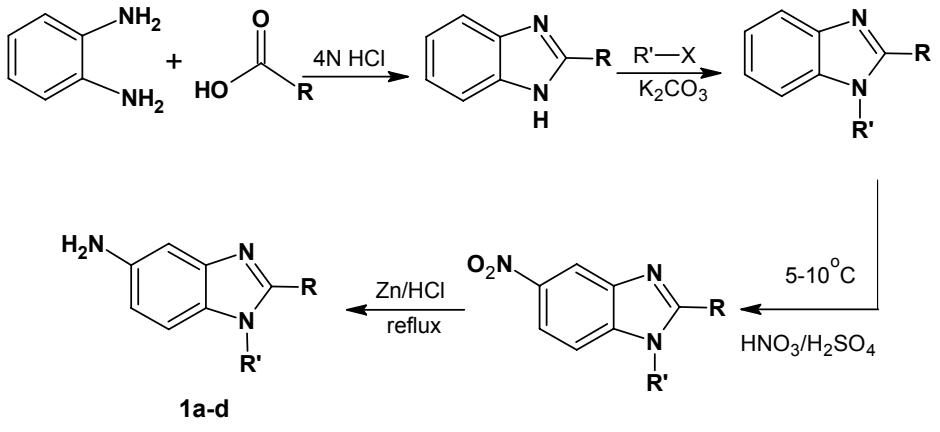
Compound **2c** revealed a triplet of 3H at 1.26 and a multiplet of 2H at 2.31 corresponding to ethyl group at C-2, in its $^1$H-NMR spectrum. A doublet of H-4 and a doublet of doublet for H-8` were exhibited at 8.46 and 7.27 ppm, respectively. Two separate multiplets at 7.77-7.60 for 3H and 7.51-7.40 ppm for 4H was also there in its $^1$H-NMR spectrum. A broad singlet at 4.64 is assigned to N-H. $^{13}$C NMR of compound **2c** showed two aliphatic carbons along with seventeen aromatic carbons in accord with molecular skeleton of *5-(2′-hydroxynaphthyl diazo)-2-ethyl benzimidazole* (**2c**).

$^1$H-NMR spectrum *5-(2′-hydroxynaphthyl diazo)-2-propyl benzimidazole* **2d** was almost comparable with **2c**. An additional multiplet of 2H observed at 1.78 along with 1.96 (m, 2H) and 1.08 ppm (t, 3H) due to the presence of propyl group at C-2 instead of ethyl in **2c**. All nine aromatic protons were observed in the range of $\delta_H$ 7.11-8.19 ppm and their corresponding carbons were exhibited in the range of $\delta_C$ 101.34-152.13 ppm.

Diazotized derivatives of benzimidazol-2-one **4a-b** were also prepared from their corresponding amino derivatives in same pattern. Structures of both derivatives **4a-b** are confirmed by mass, NMR and IR spectral data. $M^{+\bullet}$ peak observed as base peak at *m/z* = 332 for **4a** and at 304 for **4b**, confirmed their expected molecular formulae. The presence of major functional moieties were also confirmed by their IR spectral data, where absorption at 1513 and 1518 cm$^{-1}$ is for N=N, at 1616 and 1611 cm$^{-1}$ for C=N and 3050 and 3122 cm$^{-1}$ is for –OH group, whereas carbonyl of benzimidazolone absorbed at 1693 and 1681 cm$^{-1}$ respectively.

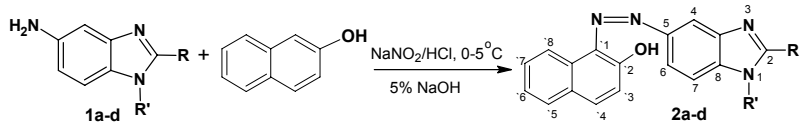
$^1$H-NMR of compound **4a** revealed that H-4 resonated at low field and appeared as a *doublet* at 8.73 ppm. H-3` appeared at 7.79 ppm ($J$ = 9.2 Hz). Another doublet of one proton at 7.72 ppm was assigned to H-6. A multiplet corresponding to three protons at 7.61 ppm has been assigned to H-4′, H-6′, H-7′ while a doublet of triplet at 7.44 ppm is assigned to H-5′. H-8′ appeared as a doublet of doublet at 7.06 ppm ($J$ = 8, 3.6 Hz). Two methyl groups at position 1 and 3 gave two singlets at $\delta_H$ 3.54 ppm and 3.48 ppm, while resonated at $\delta_C$ 28.1 and 27.7 in $^{13}$C NMR spectrum. All other seventeen carbons present in the molecule were also confirmed by $^{13}$C NMR spectrum of **4a**.

Structure of *5-(2′-hydroxynaphthyl diazo) benzimidazol-2-one* (**4b**) is also supported by $^1$H and $^{13}$C NMR, as all nine aromatic protons exhibited almost same splitting pattern as **4a,** in the range of $\delta_H$ 7.06 to 8.51 ppm, whereas seventeen carbons observed between $\delta_C$ 102.0-154.8 ppm, further confirmed the molecular skeleton of the compound **4b**.



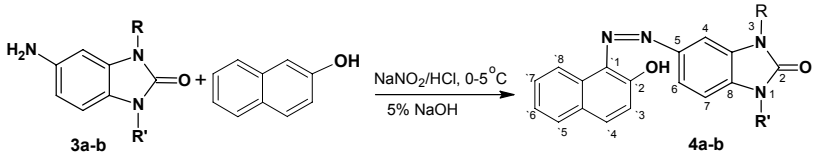

**1a-d**

Fig. 1: Synthesis of *5-Amino-2-alkyl benzimidazole* **(1a-d)**.




| | R | R′ |
|---|---|---|
| a | $CH_3$ | $CH_3$ |
| b | H | $CH_3$ |
| c | $C_2H_5$ | H |
| d | $C_3H_7$ | H |

Fig. 2: Synthesis of *5-(2′-hydroxynaphthyl diazo)-2-alkyl benzimidazole* **(2a-d)**.




| | R | R′ |
|---|---|---|
| a | $CH_3$ | $CH_3$ |
| b | H | H |

Fig. 3: Synthesis of *5-(2′-hydroxynaphthyl diazo)benzimidazol-2-one* **(4a-b)**.

*Antibacterial Activity*

All newly synthesized diazotized derivatives **2a-d** and **4a-b** were screened for antibacterial activity by Agar Well Diffusion method [40] against three standard bacterial strains, i.e., *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. Benzimidazole derivatives **2a-d** showed good activity against tested microorganisms with MIC value of 9-29 µg/ml, whereas benzimidazolone analogues **4a-b** also exhibited significant potency. Compound **2d** was found active to inhibit *S. aureus,* while **4b** eradicate *E. coli*, more efficiently. Antibacterial activities of (**2d** and **4a-b**) were found comparable with the standard drug imipenem. Results (Table-1, Chart-1) are compared with our previous report [33], which helped to deduce that antibacterial activity of 5-amino derivatives of azaheterocycles was significantly enhanced in the result of diazotization. Overall, benzimidazolone class was found more active than benzimidazole, may be due to the presence of lactam linkage.

Table-1: Antibacterial Activity of Compounds 2a-d and **4a-b**.

| Compounds | MIC/µg/ml | | |
|---|---|---|---|
| | *Escherichia coli* | *Bacillus subtilis* | *Staphylococcus aureus* |
| 2a | 14 | 22 | 17 |
| 2b | 16 | 25 | 29 |
| 2c | 11 | 21 | 14 |
| 2d | 10 | 13 | 9 |
| 4a | 13 | 14 | 11 |
| 4b | 8.5 | 13.5 | 12 |
| Imipenem | 14 | 16 | 13 |

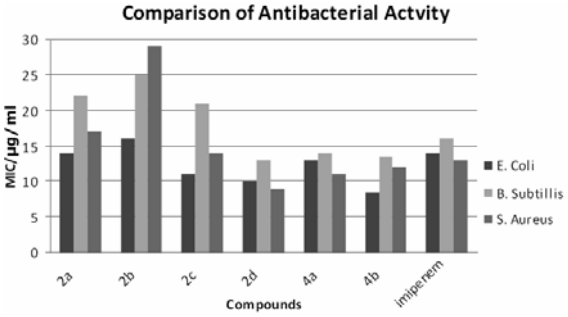



Chart-1: Comparison of Antibacterial Activity of Synthesized Compounds.

## Experimental

*General Experimental Conditions*

All the chemicals and solvents used are of analytical grade. All the reactions were monitored by thin layer chromatography using precoated (silica gel $60F_{254}$) aluminium sheets (0.2 mm layer thickness). Melting points of the synthesized compounds were recorded at IA-9100 Electrothermal Digital Melting Point Apparatus and uncorrected. $^{1}H$ and $^{13}C$ NMR spectra were recorded on Bruker Avance at 400 and 100 MHz respectively. Infrared spectra of the synthesized compounds were recorded at Shimadzo IR Spectrophotometer. Mass spectra were recorded on a double-focusing mass spectrometer (Varian MAT 311 A).

*General Procedure for Diazotization*

5-Amino benzimidazole or benzimidazolone (0.01mol) was dissolved in conc. HCl (10mL) and diluted with water (8mL) and cooled on ice bath at 0-5 $^{o}$C. The cooled solution of sodium nitrite (0.01mol) in water (10 mL) and 2-naphthol (0.01mol) in 5% NaOH (10mL) solution was added dropwise to the reaction mixture. The precipitates formed were filtered, washed with distilled water, dried and recrystallized in acetone and petroleum ether.

*5-(2′-hydroxynaphthyl diazo)-1,2-dimethyl benzimidazole (2a)*

Yield: 79%; m.p. 161 $^{o}$C; $R_f$ = 0.34 (Acetone: Pet. ether 4:1); IR (KBr, cm$^{-1}$): 3415 (OH), 1698 (C=N), 1510 (N=N); $^{1}$H-NMR (400 MHz, DMSO, δ/ppm): 8.57 (d, *J* = 3.8Hz, 1H, H-4), 7.81 (d, *J* = 6.9 Hz, 1H, H-3′), 7.76 (dd, 1H, *J* = 6.6, 3.7Hz, H-6), 7.65 (d, *J* = 6.2Hz, 1H, H-7), 7.59 (m, *J* = 8.4,1.3Hz, 3H, H-4′, H-6′, H-7′), 7.40 (dt, *J* = 8.1,1.2Hz, 1H, H-5′), 7.16 (dd, *J* = 8.1, 3.4Hz, 1H, H-8′), 3.80 (s, 3H, N-$CH_3$), 2.64 (s, 3H, C-$CH_3$); $^{13}$C NMR (125 MHz, DMSO, *δ*/ppm): 13.87, 158.56, 29.74, 136.74, 102.99, 147.6, 123.0, 119.25, 121.2, 140.4, 150.7, 121.3, 135.8, 124.9, 128.5, 127.9, 127.0, 123.2, 120.4; MS (*m/z*, %): $M^{+\bullet}$ = 316 (32), 172 (26), 191(10), 161 (100), 160 (24), 146 (43), 132 (8), 105 (19).

*5-(2′-hydroxynaphthyl diazo)-1-methyl benzimidazole (2b)*

Yield = 77%; m.p. 128 $^{o}$C; $R_f$ = 0.35 (Acetone: Pet. ether 4:1); IR (KBr, cm$^{-1}$): 3425-3295b (OH), 1685 (C=N), 1504 (N=N); $^{1}$H-NMR (400 MHz, DMSO, δ/ppm): $^{1}$H-NMR (400 MHz, DMSO, δ/ppm): 8.34 (d, *J* = 3.5Hz, 1H, H-4), 7.78 (d, *J* = 6.6 Hz, 1H, H-3′), 7.71-7.55 (m, 5H, H-6 H-7, H-4′, H-6′, H-7′), 7.38 (dt, *J* =7.9,1.1Hz 1H, H-5′), 7.14 (dd, *J* = 8, 3.2 Hz, 1H, H-8′), 3.81 (s, 3H, N-$CH_3$); $^{13}$C NMR (125 MHz, DMSO, *δ*/ppm): 159.5, 28.7, 135.9, 102.4, 148.1, 120.0, 122.1, 139.8, 152.7, 124.3, 135.0, 124.8, 127.5, 124.9, 125. 6, 126.2, 122.5, 132.3; MS: *m/z* (%): $M^{+\bullet}$ = 302 (2), 158 (31), 143 (8.5), 142 (100), 130 (5.7), 115 (4.6), 114 (13), 103 (2).

*5-(2′-hydroxynaphthyl diazo)-2-ethyl benzimidazole (2c)*

Yield = 72%; m.p. 152 $^{o}$C; $R_f$ = 0.31 (Acetone: Pet. ether 4:1); IR (KBr, cm$^{-1}$): 3190-3010 (OH), 1695 (C=N), 1545 (N=N) 1083 (C-N); $^{1}$H-NMR (400 MHz, DMSO, *δ*/ppm): 8.46 (d, *J* =3.4Hz, 1H, H-4), 7.77-7.60 (m, 3H, H-3′, H-6, H-7), 7.51-7.40 (m, 4H, H-4′, H-5′, H-6′, H-7′), 7.27 (dd, *J* = 8, 3.5Hz, 1H, H-8′), 2.31 (m, 2H), 1.26 (t, *J* = 6.8 Hz, 3H, -$CH_3$); $^{13}$C NMR (125 MHz, DMSO, *δ*/ppm): 13.7, 21.4, 158.6, 136.5, 102.6, 147.6, 119.2, 121.3, 141.5, 152.4, 147.3, 130.5, 121.5, 127.3, 126.9, 128.3, 126.1, 132.8, 120.5; MS (*m/z*, %): $M^{+\bullet}$ = 316 (100), 191(39), 172 (19), 161 (58), 160 (63), 146 (28), 133 (11), 132 (9), 106 (10), 105 (6).

*5-(2′-hydroxynaphthyl diazo)-2-propyl benzimidazole (2d)*

Yield = 81 %; m.p. 143 $^{o}$C; $R_f$ = 0.29 (Acetone: Pet. ether 4:1); IR (KBr, cm$^{-1}$): 3220, 3070 (OH), 1663 (C=N), 1507(N=N), 1041 (C-N); $^{1}$H-NMR (400 MHz, DMSO, δ/ppm): 8.19 (d, *J* = 3.5 Hz, 1H, H-4), 7.79 (d, *J* = 3.6 Hz, 1H, H-3′), 7.65-7.55 (m, 2H, H-6, H-7), 7.51 (dt, *J* = 8.5,1.1Hz 3H, H-4′, H-6′, H-7′), 7.36 (dt, *J* = 8.1,1.2Hz 1H, H-5′), 7.11 (dd, *J* = 8.1, 4.1Hz 1H, H-8′), 4.89 (bs, 1H, N-H), 1.96 (m, 2H), 1.78 (m, 2H), 1.08 (t, *J* = 6.9 Hz, 3H, -$CH_3$); $^{13}$C NMR (125 MHz, DMSO, *δ*/ppm): 15.1, 22.3, 20.6, 160.2, 137.4, 103.1, 148.2, 119.3, 120.9, 142.2, 153.4, 133.7, 132.2, 121.9, 127.6, 127.2, 127.5, 126.4, 134.1, 121.3; MS (*m/z*, %): $M^{+\bullet}$ = 330(12), 186 (23), 175 (100), 146 (69), 119 (77).

*1,3-Dimethyl-5-(2′-hydroxynaphthyl diazo)benzimidazol-2-one (4a)*

Yield = 85%; m.p. 183 $^{o}$C; $R_f$ = 0.58 (Acetone: Pet. ether 4:1); IR (cm$^{-1}$): 3050b (OH), 2995 (N-$CH_3$), 1693 (C=O), 1616 (C=N), 1518 (N=N); $^{1}$H-NMR (400 MHz, DMSO): δ(ppm): 8.73 (d, 1H, *J* = 4.3 Hz, H-4), 7.79 (d, 1H, *J* = 9.2 Hz, H-3′), 7.72 (d, 1H, *J* = 7.4 Hz, H-6), 7.61 (m, 3H, H-4′, H-6′, H-7′), 7.44 (dt, 1H, *J* =1.2, 8.2 Hz H-5′), 7.17 (d, 1H, *J* = 9.2 Hz, H-7), 7.06 (dd, 1H, J = 8, 3.6 Hz, H-8′), 3.54 (s, 3H, $CH_3$), 3.48 (s, 3H, $CH_3$); $^{13}$C NMR (125 MHz, DMSO, *δ*/ppm): 156.4, 28.1, 27.7, 105.3, 146.5, 116.8, 102.2, 129.0, 127.9, 151.9, 133.2, 130.3, 122.5, 128.1, 127.3, 128.7, 126.4, 134.2, 120.6; MS: *m/z* (%): $M^{+\bullet}$ = 332 (100), 304 (9.7), 276 (4.7), 189 (4), 162 (17), 161 (38), 147 (4), 133 (4).

*5-(2′-hydroxynaphthyl diazo)benzimidazol-2-one (4b)*

Yield = 81%; m.p. 179 $^{o}$C; $R_f$ = 0.51 (Acetone: Pet. ether 4:1); IR (cm$^{-1}$): 3122-3378 (OH and NH), 1681 (C=O), 1611 (C=N), 1513 (N=N); $^{1}$H-NMR (400 MHz, DMSO): δ(ppm): 8.51 (d, 1H, *J* = 3.9 Hz, H-4), 7.74 (d, 2H, *J* = 8.1 Hz, H-3′, H-6),

7.58 (m, 3H, H-4′, H-6′, H-7′), 7.46 (dt, 1H, *J* =1.4, 8.2 Hz H-5′), 7.06 (dd, 2H, *J* = 8.5, 3.7 Hz, H-7, H-8′). 4.81 (bs, N-H); $^{13}$C NMR (125 MHz, DMSO, $\delta$/ppm): 154.8, 105.1, 145.7, 117.4, 102.0, 129.3, 152.2, 131.9, 130.6, 121.9, 128.1, 127.7, 128.4, 125.8, 133.7, 120.4, 119.8; MS: *m/z* (%): $M^{+\bullet}$ = 304 (100), 276 (4.7), 189 (4), 162 (17), 161 (38), 147 (4), 133 (4).

*Antibacterial Activity*

Antibacterial activity of synthesized compounds was carried out against three bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*) in nutrient agar medium at concentration of 100$\mu$g/ml of DMSO by Agar Well Diffusion Method. Zone of inhibition was measured after 24 h of incubation at 37 ºC. Compounds inhibiting growth of these microorganisms were further tested for MIC (minimum inhibitory concentration). Imipenem, a broad-spectrum $\beta$-lactam antibiotic, was used as a positive control, and DMSO as a negative control.

## Conclusion

This study describe the successful synthesis of new *5-(2′-hydroxynaphthyl diazo)-2-alkyl benzimidazole* (**2a-d**) and *5-(2′-hydroxynaphthyl diazo)benzimidazol-2-one* (**4a-c**). Structures of these compounds were characterized on the basis of their spectral data. The antibacterial potential of 5-amino derivatives of reported azaheterocycles was significantly enhanced in the result of diazotization.

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