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# Synthesis and characterization of some benzothiazole substituted naphthalene derivatives and evaluation of their microbial activities

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**Abstract**: Novel compounds Benzothiazole substituted naphthalene derivatives 3(a-d), Benzothiazole naphthalene diazenyl benzenesulphonic acid derivatives 6 (a-d) and Amino benzothiazol naphthalene derivatives 7 (a-d) were synthesized according to established protocols. Characterization of these compounds were performed on the basis of FT-IR, $^1$HNMR, and elemental analysis. In addition these compounds were assayed in vitro for assessment of their microbial activities. These compounds were found to exhibit significant biological activity against both gram negative and gram positive bacterial species as well as fungal microorganisms.

**(Keywords :** Benzothiazole, Naphthalene, Diazenyl, Synthesis, Characterization, Microbial activity.)

## Introduction

Thiazole and Imidazole rings are important structural component of many biologically active molecules possessing properties of interest within the agrochemical and pharmaceutical industries. Examples include compounds exhibiting antianthrosclerotic[1], antihypertensive[2], antiinflammatory[3,4], antimicrobial[5], antioxidant[6], antiprotozoal[7], antipsychotic[8], antitumoural[9,10], antiulcerogenic[11], cardiotonic[12], fungicidal[13,14], herbicidal[15], hypocholesterolemic[16], immunosuressive[17] and insecticidal[18] activities. Moreover azole types of compounds namely thiazoles and oxazoles have been identified as molecular systems undergoing the excited state intramolecular proton transfer[19-23]. Present investigation describes the synthesis of title compounds (3,6,7), their characterization through spectroscopic methods and elemental analysis. Having achieved successful synthesis of these compounds a systematic investigation regarding their anti-microbial properties has been carried out.

## Experimental

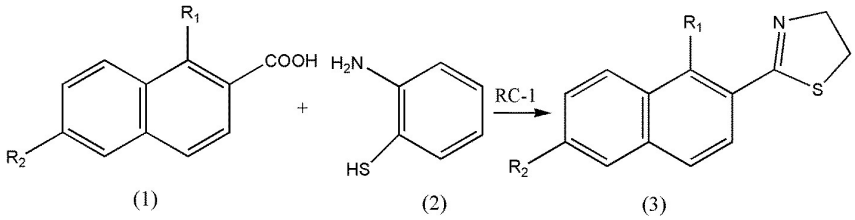
**(a) Materials and Methods :** All the reagents used were of AR Grade. 2-Naphthoic acid, 1-hydroxy-2-naphthoicacid,6-methyl-2-naphthoic acid, 2-aminothiophenol and 4-amino benzenesulphonic acid, phosphorus trichloride, sodium hydrosulfite, sodium carbonate and sodium bicarbonate were purchased from Sigma-Aldrich and used as received. 6-methoxy-2-naphthoic acid was purchased from Alfa-Aeser (Fischer Scientific) and used as received.Toluene, DMSO were purchased from local supplier for CDH, New Delhi, India and dried according to standard protocols before use. Absolute ethanol, methanol, isopropanol and benzene was procured from departmental store of the college, distilled and dried before use.

Melting points were recorded in open capillary and are uncorrected. Elemental analysis was obtained using Perkin Elmer (USA) 2400 series II CHN analyser. The IR spectra are recorded on a NICOLET-400 D spectrophotometer. $^1$H NMR spectra are obtained in $CDCl_3$ at 400 MHz on a FT-NMR, R-1500 spectrophotometer and chemical shift ($\delta$) are expressed with respect to TMS as internal standard.

**(b) Synthesis :** For synthesis of title compounds the method reported in literature[24] was reproduced with slight modifications. Reaction schemes (I, II, III) are outlined in Fig (1,2,3), Spectral data are summarized in table (1), elemental analysis, yield analysis and melting points are mentioned in table (2). General methods of synthesis for each step is as mentioned bolow :

**Step(I) : Synthesis of Benzothiazole substituted naphthalene derivatives 3(a-d) :**

A preheated two neck 250 mL round bottom flask equipped with a magnetic stirring bar was flushed twice with nitrogen. Placed 30 mL of toluene and charged with 0.05 mole of respective 2-naphthoic acid and sealed with a septum. 0.05 mole 2-aminothio phenol was introduced with the help of a glass syringe. The flask was maintained below 20°C and stirred for 1 hr. Phosphorus trichloride (0.01 mol) was transferred through syringe and stirred for another 1 hour, allowed to stabilize at ambient temperature. Finally the reaction mixture was subjected to reflux and temperature was maintained around 100°C. Progress of reaction was monitored by TLC using isopropanol and benzene in suitable proportions. The completion of reaction was indicated between four to five hours then allowed to stand overnight at room temperature. Excess of solvent was removed by vacuum distillation and the concentrated mass was triturated with saturated sodium bicarbonate solution till the pH reached 8.0. The separated solid was filtered and dried in vacuum, yield (62-71%).




**Scheme-I** : Synthesis of benzothiazol naphthalene derivatives(3).

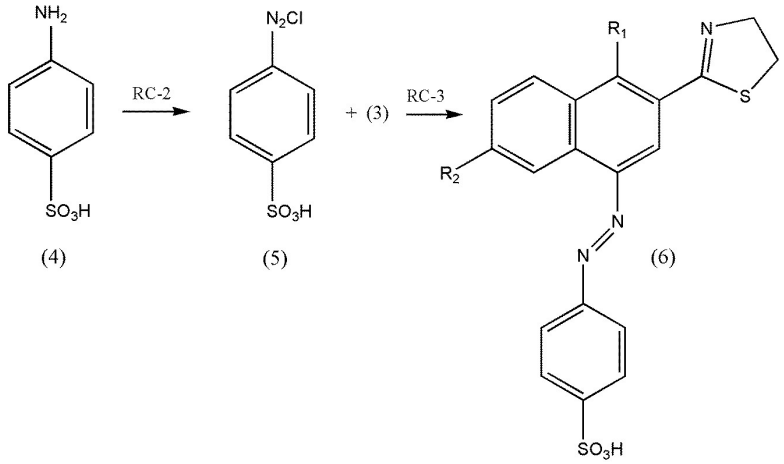
RC-1 : $PCl_3$ / Toluene, Reflux

3(a) : $R_1 = R_2 = H$ ; 3(b) : $R_1 = OH$, $R_2 = H$; 3(c) : $R_1 = H$, $R_2 = CH_3$; (d) : $R_1 = H$, $R_2 = OCH_3$

**Figure-1**

**Step(II) : Synthesis of Benzothiazole naphthalene diazenyl benzenesulphonic acid derivatives 6 (a-d) :** In 30 mL of water added 0.02 mole sodium carbonate followed by 0.02 mole sulphanilic acid and warmed with stirring until a clear solution obtained. Cooled to 5 °C and ice-cooled solution of sodium nitrite (0.02 mole in 10 mL of water) was added to the reaction mixture prepared just. The entire resulting mixture was added drop wise to 15 mL of 35% hydrochloric acid maintaining the temperature around 0-5 °C with constant stirring. It contains the diazotized sulphanilic acid (5).

0.02 mol of 3 (a-d) was added in small portions with efficient stirring to 25 mL of methanol and 10 mL double distilled water containing dissolved sodium hydroxide (0.08g, 0.02 mol). During the whole addition the temperature was maintained below 5°C. A homogeneous, pale yellow to yellow brown solution was obtained which was added with constant stirring to the diazotized sulphanilic acid (5) in small portios and under ice-cold conditions. After complete mixing the pH was preferably maintained between 9-10 with the help of saturated sodium bicarbonate solution. Within 30-45 minutes the dye 6(a-d) was separated out completely. Filtered, dried in vacuum, yield (72-84%).



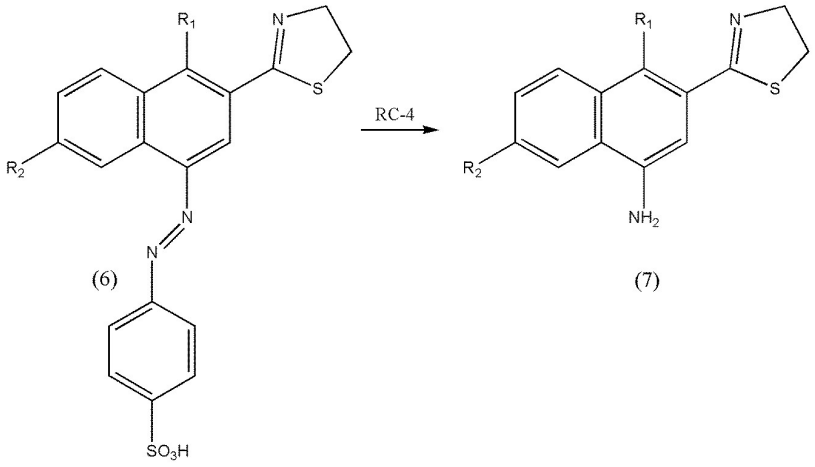

**Scheme-II** : Synthesis of naphthalene diazenylbenzenesulphonic acid derivatives(6).

RC-2 : $NaNO_2$ / HCl, 0-5 $^oC$ ; RC-3 : pH = 8-9 / 0-5$^oC$

6(a) : $R_1 = R_2 = H$ ; 6(b) : $R_1$ =OH, $R_2$ = H; 6(c) : $R_1$ = H, $R_2 = CH_3$; 6(d) : $R_1$ = H, $R_2 = OCH_3$

**Figure-2**

**Step(III) : Synthesis of Amino benzothiazol naphthalene derivatives 7 (a-d) :** 0.01 mol of 6(a-d) was dissolved in aqueous methanol (10 mL, 20%, v/v) and warmed to 50$^o$C. To this added sodium hydrosulphite (0.01 mol) in portions and the alkaline pH (8-9) was maintained by dropwise addition of saturated sodium bicarbonate solution. The dye-colour was completely discharged and compound 7(a-d) separated out. Filtered, dried yield (71-78%).




**Scheme-III** : Synthesis of Amino benzothiazolenaphthalene derivatives (7).

7(a) : $R_1 = R_2 = H$ ; 7(b) : $R_1$ = OH, $R_2$ = H; 7(c) : $R_1$ = H, $R_2 = CH_3$; 7(d) : $R_1$ = H, $R_2 = OCH_3$

RC- 4 : $Na_2S_2O_4$ / alkaline pH, 60-65 $^oC$

**Figure-3**

**Table - 1**

| Spectral Data for compounds | | |
|---|---|---|
| **Compound and Code** | **$^1$HNMR($CDCl_3$), 400MHz δ (ppm), TMS** | **FT- IR (KBr) ν ($cm^{-1}$)** |
| 3(a) : 2-(1,3-benzothiazol-2-l)naphthalene (BTN) | δ : 7.46-8.38 (m,11H,ArH) | 2295 (Ar C-H), 1655 (C=N), 1480 (Ar C-C), 782 (C-S). |
| 3(b) : 2-(1,3-benzothiazol-2-yl)-1-hydroxyl naphthalene (BTHN) | δ : 7.82-8.41 (m,10H,ArH); 4.83 (s, 1H, OH) | 3440 (O-H),3018 (Ar C-H), 1628 (C=N),1540 (Ar C-C), 1295 (Ar C-O), 758 (C-S). |
| 3(c) : 2-(1,3-benzothiazol-2-yl)-6-methyl naphthalene (BTMN) | δ : 2.54 (s,3H,$CH_3$); 7.42-8.30 (m,10H,Ar-H) | 3040 (Ar C-H),1660 (C=N), 1520 (Ar C-C), 1395 (Me C-H), 768 (C-S) |
| 3(d) : 2-(1,3-benzothiazol-2-yl)-6-methoxy naphthalene (BTMON) | δ : 3.80 (s,3H,OMe); 7.94-8.32 (m,10H,Ar-H) | 3080 (Ar C-H), 1685 (C=N), 1545 (Ar C-C), 1260 (OMe C-H), 772 (C-S) |
| 6(a) : 4-{(E)-[3-(1,3-benzothiazol-2-yl) naphthalene -1-yl] diazenyl} benzenesulphonic acid (BDBS) | δ : 2.15 (s,1H,$SO_3H$); 7.76-8.58 (m,13H,Ar-H) | 3011 (Ar C-H), 1640 (C=N), 1572 (N=N), 1485 (Ar C=C), 752 (C=S). |
| 6(b) : 4-{(E)-[3-(1,3-benzothiazol-2-yl)-1-hydroxynaphthalene-1-yl] diazenyl}benzene sulphonic acid (BHDBS) | δ : 2.10 (s,1H,$SO_3H$); 4.64 (s,1H,OH); 7.72-8.92 (m,13H,Ar-H) | 3418 (O-H), 3040 (Ar C-H), 1636 (C=N), 1560 (N=N), 1528 (Ar C-C), 1270 (Ar C-O), 782(C-S). |
| 6(c) : 4-{(E)-[3-(1,3-benzothiazol-2-yl)-7-methylnaphthalene-1-yl] diazenyl} benzenesulphonic acid (BMDBS) | δ : 2.18 (s,1H,$SO_3H$); 2.65 (s,3H,Me); 7.32-8.68 (m,13H,Ar-H) | 2990 (Ar C-H), 1664 (C=N), 1582 1572(N=N), 1510 (Ar C-C), 1380 (Me C-H ), 765 (C-S). |
| 6(d) : 4-{(E)-[3-(1,3-benzothiazol-2-yl)-7-methoxynaphthalene-1-yl] diazenyl}benzenesulphonic acid (BMODBS) | δ : 2.10 (s,1H,$SO_3H$); 3.71 (s,3H,OMe); 7.46-8.74 (m,13H,Ar-H) | 3030 (Ar C-H), 1670 (C=N), 1554 (N=N), 1528 (Ar C-C), 1380 (OMe C-H), 1274 (Ar-O), 780 (C-S). |
| 7(a) : 1-amino-3-(1,3-benzothiazol-2-yl) naphthalene (ABN) | δ : 1.58 (s,2H,$NH_2$); 7.62-8.44 (m,10H,Ar-H) | 3295 (N-H,d), 3080 (Ar C-H), 1675 (C=N), 1540 (Ar C-C), 1265 (Ar C-N), 795 (C-S). |
| 7(b) : 1-amino-3-(1,3-benzothiazol-2-yl)-4-hydroxynaphthalene (ABHN) | δ : 1.46 (s,2H,$NH_2$); 5.30 (s,1H, OH); 7.42-8.54 (m,9H,Ar-H) | 3340 (N-H,d), 3260 (O-H), 3010 (Ar C-H), 1685(C=N), 1520 (Ar C-C),1280 (Ar-O), 1240 (Ar C-N), 780 (C-S). |
| 7(c) : 1-amino-3-(1,3-benzothiazol-2-yl)-6-methylnaphthalene (ABMN) | δ : 1.54 (s,2H,$NH_2$); 2.41 (s,3H,Me); 7.54-8.36 (m,9H,Ar-H) | 3316 (N-H,d), 3110 (Ar C-H), 1675 (C=N), 1372 (Me C-H), 1260 (Ar C-N), 756 (C-S). |
| 7(d) : 1-amino-3-(1,3-benzothiazol-2-yl)-6-methoxynaphthalene (ABMON) | δ : 1.64 (s,2H,$NH_2$); 3.41 (s,3H, OMe); 7.54-8.36 (m,9H,Ar-H) | 3260 (N-H,d), 3120 (Ar C-H), 1635 ((C=N), 1345 (OMe C-H), 1295 (Ar-O), 1255 (Ar C-N), 784(C-S). |

**Assessment of Antimicrobial activity**

Antimicrobial activities were scanned by agar-cup method measuring the zone inhibition in mm. The newly synthesized compounds (3,6,7) were screnned in-vitro for their antibacterial activity against Gram positive species (Bacillus subtilis, Bacillous megaterium) and Gram negative species (Escherichia coli, Pseudomonas aeruginosa) while the antifungal activity was determined against Aspergillus niger and C. Albicans at a concentration of 75 µg/mL. The standard drugs used for antibacterial and antifungal activities were levofloxacin (500mg tablets) and fluconazole respectively. The solvent used was DMSO and each run was made in triplicate to ensure authenticity. Results are summarized in Table (3).

**Results and Discussion**

All the title compounds 3(a-d), 6(a-d) and 7(a-d) show characteristic IR-absorptions between (1620-1690 $cm^{-1}$) as well as (650-700 $cm^{-1}$) indicative of C=N and C-S respectively. Compounds 3(b), 6(b) and 7(b) show strong absorptions around 3200 $cm^{-1}$ corresponding to OH functional. Moderate peak around 1560 $cm^{-1}$ in 6(a-d) can be attributed to the diazenyl entity (N=N). Compounds 3(c), 6(c), 7(c) exhibit moderate to weak absorption in the range (1370-1385 $cm^{-1}$) which can be ascribed to the presence of $–CH_3$ group. Strong band around (3250-3350 $cm^{-1}$) in compounds 7(a-d) are due to the $–NH_2$ group. The $^1$HNMR spectra of all the compounds show multiplet with δ-value in the range 7.40-8.60 which can be ascribed to aromatic ring hydrogens. In addition compounds 3(b), 6(b), 7(b) also produce a singlet around δ = 4-5 and assigned due to –OH groups. More interestingly all the title compounds 6(a-d) have singlet peaks between (2.10-2.20) which is due to $–SO_3H$ group. Amino derivatives 7(a-d) have singlet peaks at verylow δ -values between (1.50-1.60). Methyl protons of 3(c), 6(c) and 7(c) all have singlet around δ-values (2.50-2.70), whereas methoxy protons of 3(d), 6(d) and 7(d) have their singlets at higher δ-values (3.40-3.70) which is expected due to strong electronegativity of O-atoms. Results are compiled in table (1) below.

**Table - 2**

**Elemental Analysis, Yield Analysis and Melting Points of Compounds**

| No. | Elemental analysis | | | | | | | | Yield (%) | mp ($^o$C) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Requires | | | | Found | | | | | |
| | % C | % H | % N | % S | % C | % H | % N | % S | | |
| 3(a) | 78.16 | 4.21 | 5.36 | 12.26 | 78.08 | 4.20 | 5.33 | 12.19 | (64 %) | (167-69) |
| 3(b) | 73.65 | 3.97 | 5.05 | 11.55 | 73.61 | 3.95 | 5.00 | 11.48 | (71 %) | (204-07d) |
| 3(c) | 78.54 | 4.73 | 5.09 | 11.64 | 78.51 | 4.70 | 5.02 | 11.58 | (62 %) | (123-24) |
| 3(d) | 74.22 | 4.47 | 4.81 | 10.99 | 74.16 | 4.42 | 4.76 | 10.84 | (68 %) | (182-84) |
| 6(a) | 62.16 | 3.15 | 9.46 | 14.41 | 61.98 | 3.12 | 9.42 | 14.36 | (72 %) | ((246-49d) |
| 6(b) | 59.86 | 3.25 | 9.11 | 13.88 | 59.80 | 3.20 | 9.08 | 13.81 | (78 %) | (232-35) |
| 6(c) | 62.74 | 3.70 | 9.15 | 13.94 | 62.71 | 3.65 | 9.11 | 13.92 | (76 %) | (>250d) |
| 6(d) | 60.63 | 3.58 | 8.84 | 13.47 | 60.59 | 3.55 | 8.78 | 13.45 | (84 %) | ( >250d) |
| 7(a) | 77.86 | 4.58 | 5.34 | 12.21 | 77.82 | 4.52 | 5.30 | 12.16 | (78 %) | (147-50) |
| 7(b) | 73.38 | 4.32 | 5.03 | 11.51 | 73.30 | 4.29 | 4.99 | 11.47 | (74 %) | (163-65d) |
| 7(c) | 78.26 | 5.07 | 5.07 | 11.59 | 78.19 | 5.01 | 5.04 | 11.52 | (71 %) | (215-20d) |
| 7(d) | 73.97 | 4.79 | 4.79 | 10.95 | 73.92 | 4.74 | 4.72 | 10.88 | (75 %) | > 250 |

Perusal of the elemental analysis results of above table (2) ensures correct synthesis of compounds and the yields too are reasonably satisfactory.

Regarding analysis of microbial activities some very interesting observations are there. Introduction of polar (-OH) group highly enhances the antibacterial activity of all the three series of compounds( entries 3b,6b,7b). Similarly the presence of hydrophobic moiety -$CH_3$ seems to be advantageous for antibacterial activity (entries 6b,7b). The diazenyl group in all the compounds (6a,6b,6c,6d) remarkably improves the antibacterial activity which is in conformity with classical concept of medicinal properties of azo-dyes. Combination of amino and methyl groups in 7c appears most favourable for the aforesaid property. Antifungal properties are also greatly influenced by the presence of –$CH_3$ and –OH groups. It can be inferred ,therefore, many of the title compounds have great potential to evolve as potent drug molecules. Results are summarized in table (3) below.

**Table - 3**

| Compound | Inhibition Zone Measured (in mm) | | | | | |
|---|---|---|---|---|---|---|
| | Bacterial Species | | | | Fungal Species | |
| | *E. coli* | *P. aerugin osa* | *B. subtilis* | *B. mega terium* | *A. niger* | *C. albi cans* |
| **3(a) BTN** | 12 | 15 | 17 | 16 | 14 | 19 |
| **3(b) BTHN** | 23 | 19 | 13 | 13 | 24 | 16 |
| **3(c) BTMN** | 14 | 12 | 21 | 20 | 20 | 26 |
| **3(d) BTMON** | 15 | 14 | 16 | 19 | 16 | 18 |
| **6(a) BDBS** | 18 | 16 | 12 | 15 | 14 | 13 |
| **6(b) BHDBS** | 22 | 19 | 18 | 18 | 19 | 12 |
| **6(c) BMDBS** | 16 | 24 | 21 | 23 | 26 | 25 |
| **6(d) BMOBDS** | 21 | 18 | 15 | 17 | 13 | 20 |
| **7(a) ABN** | 20 | 23 | 18 | 11 | 18 | 18 |
| **7(b) ABHN** | 18 | 17 | 21 | 14 | 21 | 23 |
| **7 (c) ABMN** | 24 | 26 | 14 | 21 | 19 | 17 |
| **7(d) ABMON** | 17 | 22 | 12 | 15 | 13 | 13 |
| **Levofloxacin** | 38 | 36 | 32 | 34 | 21 | 23 |
| **Fluconazole** | - | - | - | - | 39 | 28 |

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