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# MICROWAVE ASSISSTED SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEWER MANNICH BASES

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**ABSTRACT** : A series of Mannich bases **5a-5h** were synthesized by the reaction of 5–{2–(ethylthio)–1H–benzimidazol–1–yl}–methyl–1,3,4–oxadiazole–2–thione **4** with formaldehyde and appropriate amines by conventional and microwave techniques. The reaction rate and yields were enhanced under microwave irradiation technique rather than conventional technique. Structures were characterized by means of spectral data and elemental analyses. The antibacterial screening against *S. aureus, E. coli* and *P. aeruginosa* at three different concentrations revealed that compound **5f** is significantly active.
**KEYWORDS:** 2, 5–Disubstituted-1,3,4-Oxadiazole, Benzimidazole, Mannich bases, Antibacterial activity.

## INTRODUCTION

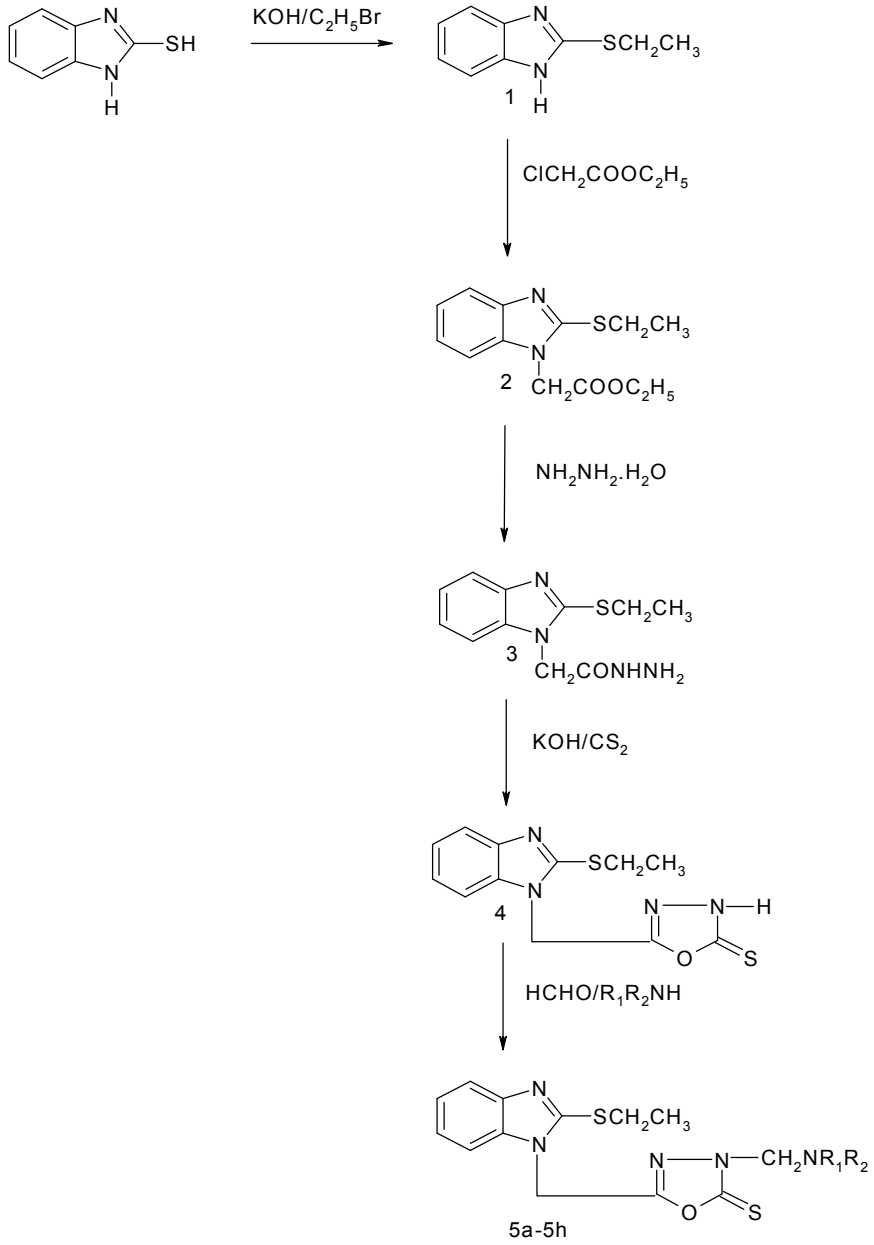
Over the past few decades, Mannich bases[1] of heterocyclic molecules have been grabbing the attention of the synthetic chemists for their wide gamut of biological activities ranging from antibacterial[2], antifungal[3], anticancer[4], antiparkinson[5] to anticonvulsant[6] and anti–HIV[7] . Also, 2,5–disubstituted–1, 3, 4–oxadiazoles[8] have also been proved to manifest various biological activities like antibacterial, antifungal[9], antimalarial, anti-inflammator[10] and anti TB[11] . In addition, benzimidazole[12] backbone also possesses a variety of potential biological activities. Therefore, expecting an enhanced bioactivity from coupling of two heterocycles, we herein report the synthesis of some Mannich bases of 1, 3, 4–oxadiazoles bearing benzimidazole moiety and their antibacterial evaluation against pathogenic microorganisms at different concentrations.

## RESULTS AND DISCUSSION

As outlined in the Scheme, 2–mercaptobenzimidazole was protected by the reaction with ethyl bromide to afford 2–ethylthiobenzimidazole **1**, which was esterified with ethylchloroacetate to yield ethyl–1–N–(2–ethylthiobenzimidazolyl) acetate **2**. Subsequent reaction of this ester with hydrazine hydrate (98%) yielded corresponding hydrazide **3** which was cyclised with carbon disulphide to obtain 5–{2–(ethylthio)–1H– benzimidazol–1–yl}–methyl–1, 3, 4–oxadiazole–2–thione **4**. The thione structure was supported by IR (KBr) spectral data as characteristic bands were observed at 3070 (NH), 1524 (C=N), 1380 (C=S), 1188, 1145, 1065 (C-O-C) $cm^{-1}$. Mannich reaction of **4** with various amines in formaldehyde at ambient temperature and at microwave yielded the desired compounds **5a-5h**. It was observed that the reaction rates and the yield manifested by the microwave technique were better compared to the conventional technique. The antibacterial evaluation of these given compounds showed that they posses moderate to good activity specifically against *S. aureus* and *E. coli* at higher concentrations only.

Spectral data (IR, NMR) and elemental analyses (C, H, N) supported the structures assigned. The physical data of compounds (**5a-5h**) are summarized in **Table 1**.

## Scheme




## BIOLOGICAL ACTIVITY[13]

All the newly synthesized Mannich bases **5a-5h** were screened for antibacterial activity against some pathogenic organisms like *S. aureus, E. coli* and *P. aeruginosa* at 50, 100 and 150 µg/ml concentrations using ampicillin as reference standard. None of these compounds showed significant activity against *E. coli* at all the three concentrations. However compounds like **5c**, **5d** and **5f** exhibited better activity against *S. aureus* at 150 µg/ml only.

The antibacterial activity was performed employing cup plate method using nutrient agar as the culture medium. The agar media were inoculated by using glass spreader technique of 0.5 mL liquid cultures. Bores of appropriate diameter were made on the medium filled with solutions of each compound of all the three concentrations. DMSO was used as the internal control. Inhibitory activity was measured (in mm) as diameter of the observed inhibition zones and the data presented in **Table 2** is the mean of readings obtained in triplicate.

## EXPERIMENTAL

### GENERAL

All the melting points were determined on 'Veego' VMP-D apparatus and are uncorrected. Silica gel G plates of 3x8 cm (Sigma-Aldrich) were used for TLC and spots were located by UV or in iodine chamber. The IR spectra were recorded in the 4000-400 $cm^{-1}$ range

using KBr discs on FTIR 8400 SHIMADZU spectrometer. $^1$H NMR spectra were recorded on Mercury (Varians) spectrometer (400 MHz) in DMSO-d6 with TMS as internal reference standard.

**Synthesis of 2 – ethylthiobenzimidazole 1 :**

To an ethanolic solution of KOH (0.12 mole, 6.75 g), 2 – mercaptobenzimidazole (0.1 mole, 15g) was added and mixed. To this, ethyl bromide (0.15 mole, 12 mL) was added drop wise and this mixture was refluxed for 8-10 hrs. The crystalline solid thus obtained was poured on ice cold water, filtered, dried and recrystallised from ethanol, yield 85%, m.p. 168 – 170$^0$C.

**Synthesis of ethyl – 1 – N – (2 – ethylthiobenzimidazolyl) acetate 2:**

2–ethylthiobenzimidazole **1** (0.1 mole, 12g), anhydrous potassium carbonate (0.1 mole, 6g) and polyethylene glycol 400 (0.1 mole, 50 mL) were added to 50 mL ethyl acetate and to this, ethyl chloroacetate (0.11 mole, 15 mL) was added drop wise and the resultant solution was stirred for 24 hrs at room temperature. It was filtered off and the filtrate was washed with water. The organic layer was distilled off to yield a semisolid compound, yield 80%; IR: cm$^{-1}$, 1750 (C=O), 750 (aromatic C-H).

**Synthesis of 2 – ethylthiobenzimidazolyl – 1 – N – methyl carbohydrazide 3:**

In the solution of **2** (0.1 mole, 9g) in methanol, was added hydrazine hydrate (99%) (0.15 mole, 2.5 mL) drop wise and heated for 7-9 hrs till TLC (ethyl acetate: hexane, 4:1) showed complete conversion. The solvent was then distilled off and residue was poured into ice cold water to yield a solid which was collected by filtration and recrystallised from ethanol, yield 70%, m.p. 120-122$^0$C, IR: cm$^{-1}$, 3285 (NH), 1658 (C=O).

**Synthesis of 5 – {2 – (ethylthio) – 1H – benzimidazol – 1 – yl} – methyl – 1,3,4 – oxadiazole – 2 – thione 4:**

In an ethanolic solution of KOH (0.15 mole, 2.3g), **3** (0.1 mole, 7g) and carbon disulphide (0.15 mole, 2.5 mL) were added. The resultant solution was refluxed till $H_2S$ gas evolution ceased. The excess solvent was distilled off and the residue was poured into ice cold water. The solution was acidified (pH 2-3) using 10% HCl and the solid thus precipitated was filtered and recrystallised from aqueous ethanol, yield 65%, m.p. 165-167$^0$C, IR: cm$^{-1}$, 3070 (NH), 1524 (C=N), 1380 (C=S), 1188, 1145, 1065 (C-O-C); $^1$HNMR: δ ppm, 7.6 – 7.3 (m, 4H, Ar), 7.22 (s, 1H, NH), 5.6 (s, 2H, N-$CH_2$), 3.4 (q, 2H, S-$CH_2$), 1.3 (t, 3H, $CH_3$).

**Synthesis of 5 – {2 – (ethylthio) – 1H – benzimidazol – 1 – yl} – methyl – 3 – {[(aryl) amino] – methyl} – 1, 3, 4 – oxadiazole – 2 – thiones (Mannich bases) 5a-5h:**

The 1,3,4-oxadiazole-2-thione **4** prepared was converted to various Mannich bases using different amines. They were prepared by both conventional as well as microwave methods as follows.

**CONVENTIONAL PROCEDURE**

To a solution of thione **4** (0.1 mole, 1g) in ethanol, was added formaldehyde (0.11 mole, 1 mL) and appropriate amine (0.11 mole,). This mixture was stirred overnight at room temperature. The product thus obtained was filtered and packed through column [silica gel, ethyl acetate: hexane (3:1)] to yield the pure compounds.

**MICROWAVE PROCEDURE**

In a solution of thione **4** (0.1 mole, 1g,) in ethanol (10 mL), were added formaldehyde (0.11 mole, 1 ml) and appropriate amines (0.11 mole). The mixture was heated in microwave at the power of 300 watts for 10 mins. The mixture was kept overnight in refrigeration. The product thus obtained was filtered and passed through column [silica gel, ethyl acetate: hexane (3:1)] to yield pure products.

**5a**: IR: cm$^{-1}$ , 3450 (NH), 1679 (C=N), 1316 (C=S), 1156, 1012 (C-O-C), $^1$HNMR: δ ppm 7.8 – 7.3 (m, 8H, Ar), 7.2 (s, 1H, NH), 5.8 (s, 2H, N-$CH_2$-N), 5.2 (s, 2H, N-$CH_2$), 3.4 (q, 2H, $CH_2$), 1.3 (t, 3H, $CH_3$). **5b**: IR: cm$^{-1}$, 3120 (NH), 1650 (C=N), 1380 (C=S), 1151, 1056 (C-O-C), $^1$HNMR: δ ppm 7.8 (s, 1H, NH), 7.6 – 6.9 (m, 8H, Ar), 5.4 (s, 2H, N-$CH_2$), 4.8 (s, 2H, N-$CH_2$-N), 3.3 (q, 2H, $CH_2$), 1.3 (t, 3H, $CH_3$).

**5c**: IR: cm$^{-1}$, 3125 (NH), 1604 (C=N), 1327 (C=S), 1193, 1113, 1086 (C-O-C), $^1$HNMR: δ ppm 7.6 – 7.4 (m, 8H, Ar), 7.0 (s, 1H, NH), 5.9 (s, 2H, N-$CH_2$-N), 5.3 (s, 2H, N-$CH_2$), 3.2 (q, 2H, $CH_2$), 1.4 (t, 3H, $CH_3$), Anal. Calcd for $C_{19}H_{18}N_6O_3S_2$: C, 51.58; H, 4.07; N, 19.00. Found C, 52.20; H, 4.43; N, 18.10. %.

**5d**: IR: cm$^{-1}$, 3100 (NH), 1519 (C=N), 1369 (C=S), 1100 (C-O-C), $^1$HNMR: δ ppm 9.3 (s, 1H, NH), 8.6 (s, 1H, OH), 7.6 – 6.6 (m, 8H, Ar), 5.6 (s, 2H, N-$CH_2$-N), 5.0 (s, 2H, N-$CH_2$), 3.4 (q, 2H, $CH_2$), 1.3 (t, 3H, $CH_3$).

**5e**: IR: cm$^{-1}$, 3150 (NH), 1690 (C=N), 1369 (C=S), 1247, 1092 (C-O-C); $^1$HNMR: δ ppm 7.9 – 7.5 (m, 8H, Ar), 7.2 (s, 1H, NH), 5.8 (s, 2H, N-$CH_2$-N), 5.3 (s, 2H, N-$CH_2$), 3.3 (q, 2H, $CH_2$), 2.3 (s, 3H, $CH_3$), 1.3 (t, 3H, $CH_3$); Anal. Calcd for $C_{19}H_{17}Cl_2N_5OS_2$: C, 48.92; H, 3.64; N, 15.02. Found C, 49.01; H, 3.60; N, 15.00. %.

**5f**: IR: cm$^{-1}$, 3150 (NH), 1610 (C=N), 1320 (C=S), 1250, 1175 (C-O-C), $^1$HNMR: δ ppm 7.7 (s, 1H, NH), 7.5 –7.1 (m, 8H, Ar), 5.5 (s, 2H, N-$CH_2$), 4.8 (s, 2H, N-$CH_2$-N), 3.8 (q, 2H, $CH_2$), 1.4 (t, 3H, $CH_3$); **5g**: IR: cm$^{-1}$, 3350 (NH), 1626 (C=N), 1375 (C=S), 1172 (C-O-C), Anal. Calcd for $C_{20}H_{21}N_5OS_2$: C, 58.39; H, 5.10; N, 17.03. Found C, 58.30; H, 5.14; N, 17.02 %.

**5h**: IR: cm$^{-1}$ 1650 (C=N), 1350 (C=S), 1167, 1119 (C-O-C), $^1$HNMR: δ ppm 7.6 – 7.2 (m, 4H, Ar), 5.6 (s, 2H, N-$CH_2$), 4.9 (s, 2H, N-$CH_2$-N), 3.8 (t, 4H, $CH_2$), 3.4 (q, 2H, $CH_2$), 2.6 (t, 4H, $CH_2$), 1.3 (t, 3H, $CH_3$).

**CONCLUSION**

The antibacterial activity revealed that mere benzimidazol-1,3,4-oxadiazole-2-thione **4** did not possess significant activity. But its conversion to Mannich bases **5a-5h** enhanced the antibacterial activity profile. 4-substituted compounds **5a** and **5d** exhibited moderate activity against *S. aureus* at high concentration. However 2, 4-disubstituted compound **5f** showed excellent activity against all bacteria at high concentration. 2-disubstituted and 3-substituted compounds **5b, 5e** and **5g** displayed

average activity. Aliphatic compound **5h** proved to be inactive in this series, stressing the fact that aromatic substitution is necessary for exhibiting some biological activity.

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**TABLE 1: PHYSICAL DATA OF COMPOUNDS 5a – 5h**

| COMPD | $R_1$ | $R_2$ | MOL. FORMULA (MOL. WT.) | m.p. ($^0$C) | CONVENT-IONAL YIELD (%) | MICRO-WAVE YIELD (%) |
|---|---|---|---|---|---|---|
| **5a** | H | 4-Fluorophenyl | $C_{19}H_{18}FN_5OS_2$ (415) | 90 – 92 | 50 | 80 |
| **5b** | H | 3- Chlorophenyl | $C_{19}H_{18}ClN_5OS_2$ (431.5) | 108 – 110 | 56 | 75 |
| **5c** | H | 4- Nitrophenyl | $C_{19}H_{18}N_6O_3S_2$ (442) | 129 – 131 | 61 | 78 |
| **5d** | H | 4-Hydroxyphenyl | $C_{19}H_{19}N_5O_2S_2$ (413) | 140 – 142 | 54 | 74 |
| **5e** | H | 2, 6-Dichlorophenyl | $C_{19}H_{17}Cl_2N_5OS_2$ (466) | semisolid | 58 | 76 |
| **5f** | H | 2 – Chloro – 4 – flurophenyl | $C_{19}H_{17}ClFN_5OS_2$ (449.5) | 120 – 122 | 58 | 77 |
| **5g** | H | 3-Methylphenyl | $C_{20}H_{21}N_5OS_2$ (411) | semisolid | 53 | 76 |
| **5h** | Morpholinyl | | $C_{17}H_{21}N_5O_2S_2$ (391) | semisolid | 60 | 79 |

**TABLE 2: ANTIBACTERIAL ACTIVITY** #

| COMPD. NO. | DIAMETER OF ZONE OF INHIBITION IN mm* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***S. aureus*** | | | ***E .coli*** | | | ***P. aeruginosa*** | | |
| | **50 #** | **100** | **150** | **50** | **100** | **150** | **50** | **100** | **150** |
| **4** | - | - | 10 | - | - | 8 | - | - | 8 |
| **5a** | 3 | 7 | 15 | - | 6 | 10 | - | 6 | 6 |
| **5b** | 3 | 7 | 14 | - | 6 | 12 | 4 | 6 | 9 |
| **5c** | 3 | 7 | 16 | 3 | 7 | 10 | - | 7 | 11 |
| **5d** | 7 | 10 | 17 | - | 8 | 10 | 3 | 8 | 10 |
| **5e** | 2 | 7 | 14 | - | 7 | 12 | - | - | - |
| **5f** | 7 | 12 | 17 | 4 | 7 | 12 | 3 | 8 | 12 |
| **5g** | 4 | 9 | 15 | 4 | 8 | 11 | 4 | 10 | 11 |
| **5h** | - | - | - | - | 6 | 11 | - | - | - |
| **DMSO** | - | - | - | - | - | - | - | - | - |
| **Ampici-llin** | - | 10 | 20 | - | 9 | 15 | - | 9 | 15 |

# Diameter of zone of inhibition expressed in mm.

All concentrations are taken as μg/Ml

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