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# Synthesis, Antibacterial and Antitubercular Evaluation of Some 1,3,4-Oxadiazole Analogues

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Reaction of 5-(3'-pyridyl)-$\Delta^4$-1,3,4-oxadiazole-2-thione (**2**) with appropriately N-substituted-β-chloropropionamides in aqueous KOH yielded corresponding 2-(N-substituted carboxamidomethylthio)-5-(3'-pyridyl)-1,3,4-oxadiazoles (**3**). Structures of various compounds (**3a-i**) were established by means of elemental analyses and spectral data. All of these compounds were screened for antibacterial and antitubercular activities. Antitubercular activity was done at 50 µg/mL against *Mycobacterium tuberculosis* H37Rv strain and antibacterial activity was carried out against *E. coli, S. aureus, S. typhii* and *P. aeruginosa* at the concentrations of 50 and 100 µg/mL. Compounds **3b**, **3c**, **3g**, **3h** and **3i** showed moderate to excellent antibacterial activity while all but **3f** exhibited maximum inhibition against mycobacteria.

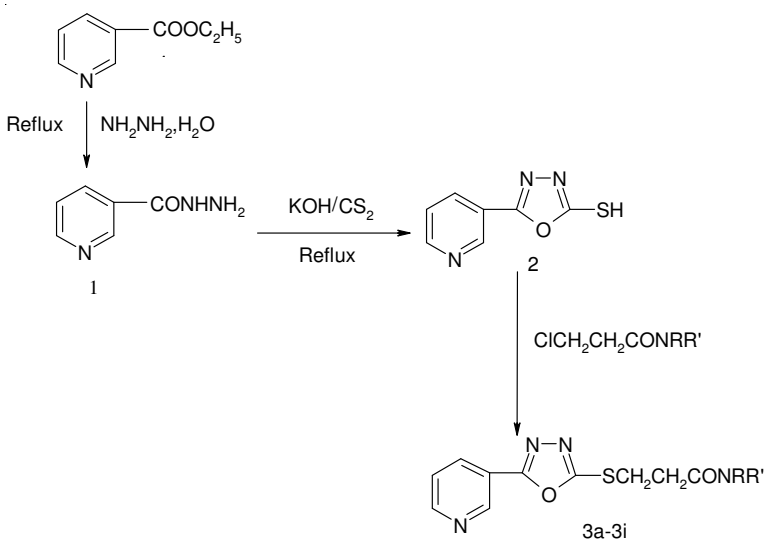
**Key Words: β-Chloropropionamides, 1,3,4-Oxadiazoles, Antibacterial, Antitubercular activity.**

## INTRODUCTION

2,5-Disubstituted-1,3,4-oxadiazole and its derivatives have been reported to possess wide spectrum of activities ranging from antibacterial[1,2], antiviral[3], plant growth regulators[4], anti-TB[5] and anti-HIV[6]. Literature has also suggested that 5-aryl/heteroaryl-2-(N-substituted carboxamidoethylthio)-1,3,4-oxadiazoles have potential antiinflammatory[7], antibacterial[8] and antitubercular activities[9]. This motivated us to couple aryl-N-substituted carboxamidoethylthio side chain with 1,3,4-oxadiazoles leading to synthesis of title compounds and their subsequent evaluation for antibacterial and antitubercular activities.

In the present work, ethyl nicotinate was synthesized[10] and subsequently converted to its hydrazide (**1**) using hydrazine hydrate (98 %). This hydrazide was refluxed in ethanol with carbon disulphide and potassium hydroxide to yield 5-(3'-pyridyl)-$\Delta^4$-1,3,4-oxadiazole-2-thione (**2**) in good yields. The substituted β-chloropropionamides were synthesized from the reaction of β-chloropropionyl chloride and corresponding aromatic/aliphatic amines in glacial acetic acid[11,12]. Then coupling of β-chloropropionamides and

thione under reflux for aromatic amides and at room temperature for aliphatic amides afforded the titled compounds (**3a-i**), which were subsequently purified by recrystallization in glacial acetic acid-water mixture (**Scheme-I**).




**Scheme-I**

## EXPERIMENTAL

Unless otherwise noted, starting materials were obtained from commercial suppliers and used without further purification. All the melting points were determined on 'Veego' VMP-D apparatus and are uncorrected. Silica gel G plates of 3 cm × 8 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 FT-IR 8400 Shimadzu spectrometer. $^1$HNMR spectra were recorded on Varian Mercury (300 MHz) spectrometer in DMSO-$d_6$ with TMS as an internal standard and values are expressed in δ ppm. The elemental analyses were performed for C, H, N at IIT-Mumbai and were within ± 0.4 % of theoretical values.

**Synthesis of pyridyl-3-carbohydrazide (1):** A mixture of ethyl nicotinate (15 g, 0.1 mol) and hydrazine hydrate (98 %) (5.1 g, 0.1 mol) was refluxed for 3 h. Absolute ethanol (25 mL) was then added and the reaction mixture was further refluxed for 1 h. The excess of solvent was removed and the residue was poured into ice cold water (100 mL).The crude solid obtained was recrystallized from ethanol to get white crystalline product (84.6 %), m.p. 161-163 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$) 3051 (CH of pyridyl), 1671 (CONH), 1631 (C=N).

**Synthesis of 5-(3'-pyridyl)-$\Delta^4$-1,3,4-oxadiazole-2-thione (2):** To a solution of pyridyl-3-carbohydrazide (**1**) (13.7 g, 0.1 mol) in ethanol (100 mL) was added a solution of potassium hydroxide (5.6 g, 0.1 mol) in water (36 mL) and stirred well. Carbon disulfide (7 mL) was then added and the mixture was refluxed till the evolution of $H_2S$ ceased. Excess of solvent was removed under vacuum and the residue poured into ice-cold water (100 mL). It was filtered to remove suspended impurities and acidified with dil. HCl to obtain the desired product. Subsequent filtration and washing with cold water afforded the product. It was recrystallized from ethanol to get colourless needles, yield 78.2 %, m.p. 231-233 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$) 3051 (CH of pyridyl), 1628 (C=N), 1355 (C=S), 1026 (C-O-C, oxadiazole).

**Synthesis of 2-(N-substituted carboxamidoethylthio)-5-(3'-pyridyl)-$\Delta^4$-1,3,4-oxadiazole (3a-i):** Compound **2** (1.79 g, 0.01 mol) was dissolved in aqueous potassium hydroxide solution (0.61 g in 10 mL water) under stirring till a clear yellow solution was obtained. It was filtered to remove any suspended impurities. Then aromatic N-substituted-β-chloropropionamides[11-13] (0.011 mol) were added in small portions with shaking at 50-60 ºC for 4-5 h and aliphatic N-substituted-β-chloropropionamides were added at room temperature. Then the reaction mixture was left overnight. The precipitate thus separated was filtered and washed twice with cold water and recrystallized from 1:1 mixture of glacial acetic acid-water. The physical and analytical characteristics of these compounds are reported in Table-1.

**3b:** $^1$H NMR (DMSO-$d_6$, δ ppm) 9.21 (s, 1H, CONH), 8.89-8.77 (dd, 2H, α-pyridyl), 8.30-8.28 (d, 1H, γ-pyridyl), 8.09-8.00 (m, 1H, β-pyridyl), 7.50-7.36 (m, 4H, aromatic), 6.88-6.85 (m, 1H, β-pyridyl), 3.98 (s, 2H, $SCH_2$), 3.86 (s, 2H, $SCH_2$), 3.42 (s, 3H, $OCH_3$).

**3d:** $^1$H NMR (DMSO-$d_6$, δ ppm) 9.28 (s, 1H, CONH), 8.88-8.87 (dd, 2H, α-pyridyl), 8.41-8.36 (d, 1H, γ-pyridyl), 8.15-8.10 (m, 1H, β-pyridyl), 7.78-7.62 (m, 4H, aromatic), 4.01 (s, 2H, $SCH_2$), 3.96 (s, 2H, $SCH_2$).

**3g:** $^1$H NMR (DMSO-$d_6$, δ ppm) 8.76-8.73 (dd, 2H, α-pyridyl), 8.38-8.31 (d, 1H, γ-pyridyl), 7.98-7.83 (m, 1H, β-pyridyl), 4.02 (s, 2H, $SCH_2$), 3.88 (s, 2H, $SCH_2$), 2.07-1.25 (m, 7H, *n*-propyl).

**3i:** $^1$H NMR (DMSO-$d_6$, δ ppm) 8.87-8.80 (dd, 2H, α-pyridyl), 8.42-8.40 (d, 1H, γ-pyridyl), 7.98-7.94 (m, 1H, β-pyridyl), 4.03 (s, 2H, $SCH_2$), 3.96 (s, 2H, $SCH_2$), 1.68-1.29 (m, 8 H, morpholinyl).

**Antibacterial activity:** All compounds were screened *in vitro* for their antibacterial activity against *E. coli, S. aureus, S. typhii* and *P. aeruginosa* using cup-plate agar diffusion method[13] at 50 µg/mL, 100 µg/mL concentrations using streptomycin as the standard and DMF as control because test compounds were dissolved into it. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zone.

TABLE-1
PHYSICAL AND ANALYTICAL DATA OF TITLED COMPOUNDS (**3a-3i**)

| Compd. | R | R′ | m.f. (m.w.) | m.p. (ºC) (Yield %) | IR ($cm^{-1}$) |
|---|---|---|---|---|---|
| **3a** | H | *p*-Fluorophenyl | $C_{16}H_{13}O_2N_4SF$ (344) | 138-140 (74) | 3198 (-NH), 2956 (-CH), 1683 (-CONH), 1412 (-$SCH_2$), 1131, 1087 (C-O-C), 878, 705 (1, 4-disubst. Ph). |
| **3b** | H | *p*-Methoxyphenyl | $C_{17}H_{16}O_3N_4S_4$ (356) | 167-169 (66) | 3217 (-NH), 3060 (-CH), 1677 (-CONH), 1420 (-$SCH_2$), 1170, 1048 (C-O-C), 846, 713 (1, 4-disubst. Ph). |
| **3c** | H | *p*-Nitrophenyl | $C_{16}H_{13}O_4N_5S$ (371) | 224-226 (67) | 3252 (-NH), 2989 (-CH), 1679 (-CONH), 1417 (-$SCH_2$), 1504 (NO2), 1184 (C-O-C), 750 (1, 4-disubst. Ph). |
| **3d** | H | *p*-Bromophenyl | $C_{16}H_{13}O_2N_4SBr$ (405) | 115-117 (88) | 3219 (-NH), 2960 (-CH), 1681 (-CONH), 1428 (-$SCH_2$), 1138, 1066 (C-O-C), 807, 715 (1, 4-disubst. Ph). |
| **3e** | H | *p*-Chlorophenyl | $C_{16}H_{13}O_2N_4SCl$ (360.5) | 89-91 (90) | 3216 (-NH), 2965 (-CH), 1687 (-CONH), 1406 (-$SCH_2$), 1160, 1080 (C-O-C), 817, 705 (1, 4-disubst. Ph). |
| **3f** | H | 2,6-Dichlorophenyl | $C_{16}H_{12}O_2N_4SCl_2$ (395) | 130-133 (75) | 3200 (-NH), 2916 (-CH), 1667 (-CONH), 1398 (-$SCH_2$), 1110, 1080 (C-O-C), 819, 735 (1, 2, 6-trisubst. Ph). |
| **3g** | H | *n*-Propyl | $C_{13}H_{16}O_2N_4S$ (292) | 147-151 (55) | 3217 (-NH), 2993 (-CH), 1692 (-CONH), 1413 (-$SCH_2$), 1117, 1080 (C-O-C). |
| **3h** | H | *n*-Butyl | $C_{14}H_{18}O_2N_4S$ (306) | 64-66 (60) | 3242 (-NH), 3045 (-CH), 1687 (-CONH), 1400 (-$SCH_2$), 1119, 1058 (C-O-C) |
| **3i** | Morpholinyl | | $C_{14}H_{16}O_3N_4S$ (320) | 156-159 (60) | 2965 (-CH), 1666 (-CONH), 1419 (-SCH2), 1116, 1067 (C-O-C) |

Compounds like **3a**, **3c**, **3g** and **3i** displayed moderate activity against almost all bacteria at both concentrations. But **3h** has exhibited an excellent antibacterial activity against all microbes, even better the standard, against *E. coli* and *P. aeruginosa*. The data of activity is summarized in Table-2.

**Antitubercular activity:** Anti tubercular activity was carried out at Tuberculosis Antimicrobial Acquisition Co-ordination Facility (TAACF), USA. The screening was done at 50 µg/mL concentration against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence are tested in the BACTEC 460-radiometric assay[14]. The activity data have been compared with the standard drug rifampin at 0.25 µg/mL concentration and presented in Table-2.

TABLE-2
ANTIBACTERIAL AND ANTITUBERCULAR ACTIVITIES OF 1,3,4-OXADIAZOLES COMPOUNDS (**3a-i**)

| Compd. | Antibacterial activity* | | | | | | | | Anti-TB** activity |
|---|---|---|---|---|---|---|---|---|---|
| | *E. coli* | | *S. aureus* | | *S. typhi* | | *P. aurogenosa* | | |
| | 50 | 100 | 50 | 100 | 50 | 100 | 50 | 100 | |
| **3a** | – | 17 | – | 18 | – | 12 | – | 19 | 97 |
| **3b** | 11 | 16 | 12 | 21 | – | 19 | – | 15 | 96 |
| **3c** | 12 | 17 | 10 | 20 | – | 19 | – | 16 | 99 |
| **3d** | – | 14 | 9 | 16 | – | 18 | 15 | 21 | 99 |
| **3e** | – | 16 | – | 14 | 12 | 20 | – | 19 | 99 |
| **3f** | – | 11 | – | 14 | – | 18 | – | 18 | 33 |
| **3g** | 14 | 19 | – | 16 | 10 | 17 | 18 | 23 | 95 |
| **3h** | 22 | 26 | 17 | 24 | 15 | 21 | 21 | 31 | 100 |
| **3i** | 14 | 19 | 13 | 20 | 11 | 19 | 17 | 24 | 99 |
| Rifampin | – | – | – | – | – | – | – | – | 100 |
| Streptomycin | 18 | 21 | 17 | 22 | 18 | 23 | 15 | 29 | – |

*Zone of inhibition (mm); ** Inhibition (%).

The result showed that these compounds have excellent anti tubercular activity. At the concentration of 50 µg/mL compounds like **3a**, **3b**, **3c**, **3d**, **3e**, **3h** and **3i** showed almost 100 % inhibition while other compounds like **3g** exhibited almost 95 % inhibition, while poor activity was seen for the compound **3f**.

## RESULTS AND DISCUSSION

All the title compounds were synthesized in quantitative yield. The oxadiazole **2** may exist in thione-thiol tautomeric forms, but the present investigation showed that in this particular case the thione structure dominates in the solid state, as indicated by the IR and NMR data. The $^1$H NMR of **2** did not exhibit any signal for SH which is generally observed[14] at around

δ ppm. The various N-substituted-β-chloropropionamides were obtained by the reaction of β-chloropropionyl chloride with corresponding mono/disubstituted aromatic/aliphatic amines in glacial acetic acid-sodium acetate mixture system at lower temperature. They were confirmed by IR (KBr) spectra which showed a sharp band in the range of 1680-1650 $cm^{-1}$ due to -CONH functionality. The target compounds **3a-i** were obtained by the coupling of thione and β-chloropropionamides and subjected to *in vitro* antibacterial and antitubercular screening.

The antibacterial screening, in general, showed that compounds substituted with both aliphatic and aromatic chloropropionamides have moderate activity. The results showed that degree of inhibition vary with the structure of the compounds. But specific conclusion can not be drawn from these results. In case of antitubercular screening, very promising results have been obtained. All of the compounds, except **3f**, have shown maximum inhibition of the mycobacteria. These findings stress the fact that these compounds are very specific towards mycobacteria and demand further investigations so as to determine minimum inhibitory concentrations (MIC).

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