Synthesis and antimicrobial studies of 2-(5-substituted)-1, 3, 4-oxadiazole-2-yl)-H-imidazo [1, 2, α] pyridine derivatives

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ABSTRACT

A novel series of 2-(5-substituted)-1,3,4-oxadiazole-2-yl)-H-imidazo[1,2,α]pyridine derivatives 5a-i and 6a-d are synthesized and characterized by IR, 1H NMR, 13C NMR and Mass spectral analysis. All the synthesized compounds were tested for there antibacterial and antifungal activity of which compound 5b, 5c, 5d, 5e, 5f, 5g, 5h, 5i, 6a, 6c and 6d exhibited good antimicrobial activity.

Keywords: Imidazo [1, 2-α] pyridine; 1, 3, 4-oxadiazole; antibacterial; antifungal activity.

INTRODUCTION


Derivatives of 1, 3, 4-oxadiazole have been found to possess a wide spectrum of pharmacological, medical and biological activities [33-34]. Compounds containing 1, 3, 4-oxadiazole nucleus also find unique place in medicinal chemistry and play significant role as they are associated with immense biological activity. The small and simple 1,3,4-oxadiazole nucleus is present in compounds involved in research aimed at evaluating new compounds that posses interesting pharmacological properties like antipanosonal, antibacterial, fungicidal, herbicidal, antitumor, anti-inflammatory, antituberculosis, diuretic, hypoglycemic, anticonvulsant and analgesic. 2, 5-disubstituted-1, 3, 4-oxadiazole derivatives have attracted considerable attention owing to their effective biological activity and extensive use.

In light of the above mentioned findings and in continuation of our work on various heterocyclic compounds as potential antimicrobial agents, we herein report the design, synthesis and antimicrobial evaluation of some novel 2,5-disubstituted 1,3,4-oxadiazole derivatives with imidazo pyridine and substituted phenyl and styryl substitutions.

EXPERIMENTAL SECTION

Melting points of compounds were determined in open capillary tubes in silicon oil bath using a Veego melting point apparatus and are uncorrected. Purity of compounds was monitored by TLC on silica F254 coated aluminum plates (Merck) as adsorbent and U.V. light and Iodine chamber as a visualizing agent. IR spectra (KBr in cm⁻¹) were recorded on Shimadzu Model FTIR-435. 1H-NMR spectra were recorded on a Varian mercury TH-300 operating at 300 MHz & 400 MHz using CDCl3 as a solvent and TMS as a internal standard (Chemical shift in ppm) and high
resonance mass spectra were made on a Waters Q-T mass spectrometer. The isotopic peak at M+2 was observed in the mass spectrum of all the compounds due to S and Cl or Br.

**Synthesis of Ethyl-2-methyl-H-imidazo[1,2-a]pyridine-3-carboxylate (3):**

2-Amino pyridine 1 (15.0 g, 1.0 mol) was dissolved in absolute ethanol (30 ml). To this potassium carbonate (26.44 g, 1.2 mol) was added and the reaction mixture was stirred for 15 min. To this mixture, ethyl-2-chloro oxobutanoate 2 (31.48 g, 1.2 mol) in ethanol (15 ml) was added drop wise and then refluxed for 8-10 hrs. After completion of reaction as monitored on TLC, the reaction was cooled at room temperature. The solid was filtered and washed with ethanol (15 ml). Ethanol was removed under reduced pressure and 50 gm crushed ice was added and reaction mass was stirred for 15 min. The product was then extracted with dichloromethane (4 x 25 ml), the combined dichloromethane was washed with saturated brine water (50 ml) and dried over sodium sulphate. Dichloromethane was removed under reduced pressure to obtain 24.37 g product.

**Synthesis of 2-methylH-imidazo[1,2-a]pyridine-3-carboxydrazide (4):**

To a mixture of compound 3 (24.0 g) in methanol (50 ml), hydrazine hydrate (48.0 ml) was added and refluxed for 4 hrs. After completion of the reaction the solid product separated out was filtered and washed with methanol (5 ml) to obtain 12.40 g pure product. Yield, 55 %; mp 187-189ºC; IR (KBr, cm⁻¹): 1244 (C-N), 1494 (C=C), 1626 (C=O), 3288 (NH); H NMR (CDCl₃, 400 Hz): δ 2.87 (s, 3H, -CH₃), 4.12 (bs, 2H, -NH₂), 6.90-6.93 (t, 1H, J = 7.2, ArH), 7.06 (bs, 1H, -NH), 7.30-7.4 (t, 1H, J = 7.2 Hz, ArH), 7.55-7.57 (d, 1H, J = 9.0 Hz, ArH), 9.30-9.32 (d, 1H, J = 6.8 Hz, ArH). ¹³C NMR (CDCl₃, 100 Hz): δ 15.40, 38.96, 39.16, 39.37, 39.58, 39.79, 40.21, 112.43, 114.42, 115.73, 126.21, 126.93, 145.12, 145.20, 161.77.

**General procedure for the synthesis of 5-(3-methyl-H-imidazo[1,2-a]pyridin-2-yl)-1,3,4-oxadiazole-2-thiol (5a-i and 6a-d):** To compound (4) (1.0 gm, 1.0 mol), phosphorus oxychloride (24.0 ml) was added. This mixture of benzonic acid or cinamic acid (1.1 mol) was then added and refluxed for 8-10 hrs. After completion of the reaction (As checked by TLC) it was poured to ice. Solid product obtained was filtered and purified by column chromatography using hexane: ethyl acetate as eluent.

**2-Methyl-3-(5-phenyl-[1,3,4]oxadiazole-2-yl)-imidazo[1,2-a]pyridine (5a):**

Yield, 78%; mp 157-159ºC; IR(KBr cm⁻¹): 1096 (C-O), 1261 (C-N), 1308 (C=C), 1655 (C=O); H NMR (CDCl₃, 400 Hz): δ 2.82 (s, 3H, -CH₃), 7.27-7.31 (m, 1H, J = 6.8 Hz, ArH), 7.55-7.59 (m, 1H, J = 8.12 Hz, ArH), 7.65-7.68 (m, 3H, J = 7.52 Hz, ArH), 7.74-7.76 (m, 1H, J = 9.0 Hz, ArH), 8.13-8.20 (m, 2H, ArH), 9.33-9.40 (m, 1H, J = 7.64 Hz, ArH). ¹³C NMR (CDCl₃, 100 Hz): 15.33, 114.22, 116.43, 123.13, 126.45, 127.29, 127.30, 131.77, 146.14, 147.97, 157.76, 161.99; HRMS (EI mode) m/z calc for C₁₅H₁₅N₂O [M+H] 277.1044 found 277.0800.

**2-Methyl-3-(5-tolyl-[1,3,4]oxadiazole-2-yl)-imidazo[1,2-a]pyridine (5b):**

Yield, 85%; mp 182-183ºC; IR (KBr cm⁻¹): 1047 (C-O), 1258 (C-N), 1602 (C=O); H NMR (CDCl₃, 400 Hz): δ 2.80 (s, 3H, -CH₃), 2.87 (s, 3H, -CH₃), 7.05-7.10 (t, 1H, J = 8.8 Hz, ArH), 7.35-7.47 (m, 4H, ArH), 7.67-7.70 (d, 1H, J = 11.0 Hz, ArH), 8.01-8.04 (d, 1H, J = 10.0 Hz, ArH), 9.52-9.54 (d, 1H, J = 9.2 Hz, ArH); ¹³C NMR (CDCl₃, 100 Hz): 15.71, 22.31, 113.90, 116.76, 126.32, 127.27, 127.95, 128.52, 131.28, 131.88, 138.43, 146.14, 147.97, 148.46; HRMS (EI mode) m/z calc for C₁₇H₁₁FN₂O [M+H] 291.0866 found 291.0769.

**3-[2-(Fluro phenyl)-[1,3,4]oxadiazole-2-yl]-2-methylimidazo[1,2-a]pyridine (5c):**

Yield, 80%; mp 198-199ºC; IR (KBr cm⁻¹): 1071 (C-O), 1257 (C-N), 1339, 1449, 1615 (C=O); H NMR (CDCl₃, 400 Hz): δ 2.80 (s, 3H, -CH₃), 2.84-2.86 (m, 3H, ArH), 7.68-7.70 (m, 2H, ArH), 7.81-7.83 (m, 1H, ArH), 8.13-8.17 (m, 1H, ArH), 9.40-9.42 (m, 1H, ArH); ¹³C NMR (CDCl₃, 100 Hz): 13.92, 106.63, 107.50, 111.39, 115.14, 115.68, 116.93, 117.13, 118.63, 125.16, 127.79, 129.39, 129.89, 134.04, 143.26, 144.10, 145.10, 151.69, 156.66, 157.22, 157.95; HRMS (EI mode) m/z calc for C₁₅H₁₁FN₂O [M+H] 295.0950 found 295.0617.

**3-[2-(Iodo phenyl)-[1,3,4]oxadiazole-2-yl]-2-methylimidazo[1,2-a]pyridine (5d):**

Yield, 76%; mp 198-199ºC; IR (KBr cm⁻¹): 1019 (C-O), 1250 (C-N), 1603 (C=O); H NMR (CDCl₃, 400 Hz): δ 2.90 (s, 3H, -CH₃), 7.09-7.15 (m, 1H, ArH), 7.22-7.26 (m, 2H, ArH), 7.39-7.47 (m, 2H, ArH), 7.51-7.55 (m, 1H, ArH), 7.73-7.76 (m, 1H, ArH), 7.92-7.99 (m, 1H, ArH), 8.01-8.08 (m, 1H, ArH); ¹³C NMR (CDCl₃, 100 Hz): 15.84, 94.15, 99.99, 107.64, 114.20, 116.72, 127.71, 127.85, 128.07, 128.42, 128.95, 131.02, 131.61, 132.16, 132.52.
3-[5-(3-Chloro phenyl)-1,3,4-oxadiazole-2-yl]-2-methyl-imidazo[1,2-a]pyridine (5d):
Yield, 75%; mp 194-197° C; IR (KBr cm⁻¹): 1244, 1626, 3217; 1H NMR (CDCl₃, 400 Hz): δ 7.77 (s, 9H, -C(CH₃)₃), 2.82 (s, 3H, -CH₃); 13C NMR (CDCl₃, 100 Hz): 14.09, 30.89, 34.58, 108.64, 114.79, 115.01, 125.16, 125.73, 127.78, 128.35, 130.22, 130.31, 133.74, 133.05, 133.85, 138.53, 148.60, 158.75, 160.74; HRMS (EI⁺ mode) m/z calcd for C₁₆H₁₂ClN₂O [M+H] 345.1828, found 345.0135.

3-[5-(4-Fluoro phenyl)-1,3,4-oxadiazole-2-yl]-2-methyl-imidazo[1,2-a]pyridine (6d):
Yield, 79%; mp 188-191° C; IR (KBr cm⁻¹): 1244, 1626 (C=C), 3217 (C=H); 1H NMR (CDCl₃, 400 Hz): δ 1.17-1.33 (s, 9H, -C(CH₃)₃), 7.73-7.75 (m, 9H, -ArH), 7.78-7.80 (m, 3H, -ArH), 7.86-7.88 (m, 1H, ArH), 9.41-9.42 (d, 1H, J= 6.8 Hz, ArH); 13C NMR (CDCl₃, 100 Hz): 14.09, 30.89, 34.58, 108.64, 114.79, 115.01, 125.16, 125.73, 127.73, 128.03, 129.09, 130.24, 131.94, 133.82, 152.90, 156.37, 162.64; HRMS (EI⁺ mode) m/z calcd for C₁₄H₁₃F₂N₂O [M+H] 359.1872, found 359.1660.
Antimicrobial Activity

The antibacterial activity of all the newly synthesized compounds was done by the Muller-Hinton agar-well diffusion assay technique. The stock solutions of all test compounds (100 µg/mL) were prepared by dissolving 100 µg of the test compound in DMSO (1 mL). Chloramphenicol and DMSO were used as positive and negative controls, respectively. Twenty milliliter of molten and cooled MHA and 320 µL of each test bacterial culture were mixed (separate flasks were used for each bacterial culture) and poured in sterilized and labeled petri plates. The wells of 6 mm were punched in the solidified petri plates, aseptically. Fifty micro litters from stock solutions of all compounds as well as controls was added to each well of labeled petri plates and incubated at 35°C for 24 h. The diameter of the zone of growth inhibition around each well was measured after incubation using vernier caliper.

The minimum inhibitory concentration (MIC) of compounds against Gram-positive and Gram-negative test bacteria was determined in the range 100 to 40 µg/mL. All the test cultures were streaked on SCDA and incubated overnight at 37°C. Stock solution of each compound was prepared in DMSO and was appropriately diluted to get a final concentration of 100, 90, 80, 70, 60, 50 and 40 µg/mL. Standard antibiotic chloramphenicol was also diluted to get a final concentration in the same manner.

For the antifungal activity, Potato dextrose agar (Hi media) medium was used. This sterilized hot medium (15 mL) was pipette out into flat petri plates. When it solidified 15 mL of warm seeded agar was applied over it. The seeded agar was made by cooling the medium to 40°C and then adding spore suspension to seeded medium. Nystatin and DMSO were used as positive and negative controls, respectively. Concentration 100 µg/mL of the synthesized compounds were prepared by dissolving the required quantity of compounds in DMSO, sterilized Whatman filter paper number 541 discs were prepared by cutting 6 mm diameter were spread individually with needle and planted upon the chilled seeded medium. The culture plates were then incubated for 24–72 h at 37°C and inhibition zone around each disc was measured from the centre of the discs. The diameter of growth inhibition zone was calculated by vernier caliper. For minimum inhibitory concentrations (MIC) of synthesized compounds were determined in the range of concentrations from 100 to 40 µg/mL.

RESULTS AND DISCUSSION

The synthesis of the target molecules was achieved as per the synthetic scheme given in Figure 1. Accordingly, 2-amino pyridine was treated with chloro-oxobutanoate to obtain compound 3 which was converted to its corresponding carbazide derivative 4 using hydrazine hydrate. Compound 4 was further reacted with either substituted benzoic acids or cinnamic acids in presence of POCl3 to afford target compounds 5a-i and 6a-d in almost quantitative yields. All the synthesized compounds were analyzed by spectroscopic analysis.

Structure of compound 3 was established by 1H NMR which showed presence of ethyl group 1.40 (-CH3) and 4.40 (-CH2-). Formation of compound 4 was confirmed by 1H NMR and IR which showed the absence of ethyl ester peaks 1.40 and 4.40 δ and presence of carbazide peaks peak 4.14 (-NH2) & 7.01 (-NH-) δ. All the target compounds were characterized by IR, 1H NMR, 13C NMR and mass spectrometric techniques. The representative 1H NMR spectrum of compound 5a shows disappearance of carbazide proton at δ 4.14 (-NH2) and 7.01 (-NH-), while disappearance of carbazide amide at 1626 cm−1 in IR confirmed the structure. Further the structure was confirmed beyond doubt by HRMS which shows the M+1 peak at 277.0800. calcd for C16H12N4O [M+H] 277.1044.

Similarly, the structures of all the other derivatives were confirmed by analytical data, the results are presented in the experimental part.
Figure 1. Synthesis of compounds 6a-k. Reagents and conditions: (a) ethanol, K$_2$CO$_3$, reflux, 10 h; (b) ethanol, NH$_4$H$_2$O$_2$, reflux, 5 h; (c) Ar-COOH,POCl$_3$, reflux, 6 h

Table 1: Antibacterial activity of the compounds (5a-i and 6a-d) as MIC (µg/ml)

<table>
<thead>
<tr>
<th>Compound</th>
<th>S.aureus</th>
<th>E.coli</th>
<th>B.subtilis</th>
<th>P.aeruginosa</th>
<th>S.pyogens</th>
<th>K.terrigena</th>
<th>K.pneumonae</th>
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<td>70</td>
<td>90</td>
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<td>80</td>
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<tr>
<td>5b</td>
<td>70</td>
<td>80</td>
<td>70</td>
<td>60</td>
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<tr>
<td>5c</td>
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<td>60</td>
<td>70</td>
<td>60</td>
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<td>50</td>
<td>70</td>
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</tr>
<tr>
<td>5f</td>
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<td>5i</td>
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<td>70</td>
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<td>80</td>
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</tbody>
</table>

| 6a       | 90      | --    | 90         | 70          | --        | --          | 90          |
| 6b       | 70      | 90    | 80         | 70          | --        | --          | --          |
| 6c       | 70      | 70    | 70         | 70          | 90        | 50          | 60          |
| 6d       | 70      | 70    | 70         | 70          | 70        | 80          | 60          |
| Chloramphenicol | 50     | 50    | 40         | 40          | 40        | 40          | 50          |

Table 2: Antifungal activity of the compounds (5a-i and 6a-d) as MIC (µg/ml)

<table>
<thead>
<tr>
<th>Compound</th>
<th>T. Viride</th>
<th>A flavus</th>
<th>A brasillansis</th>
<th>C. albicans</th>
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<td>NCIM</td>
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<td>5c</td>
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<tr>
<td>Nystatin</td>
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</table>
The newly synthesized compounds 5a-i and 6a-d were tested for their antibacterial activity against S. aureus, E. coli, B. subtilis, P. aeruginosa, S. pyogenes, K. terrigena and K. pneumoniae. All the synthesized compounds were dissolved in DMSO and antibacterial activity studied by disc diffusion method. Compound 5b, 5c, 5d, 5e, 5f, 5g, 5h, 5i, 6a, 6c and 6d shows good antibacterial activity against several species. The results are summarized in Table 1. Compound 6a-k were also tested for antifungal activity against T. viride, A. flavus, A. brasiliensis and C. albicans. Among the all synthesized compounds 5d, 5g, 5h and 6c shows good antifungal activity. The results are summarized in Table 2. It is evident from the table that there is very little effect of the various substituents [electron donating CH$_2$, electron withdrawing (NO$_2$) or halogens (F, Cl, Br)] on the antimicrobial activity. Compound 5a with no substitution also show significant activity which suggests that activity is due to oxadiazole ring clubbed with imidazo pyridine.

CONCLUSION

We have synthesized a new series of 2-(5-substituted)-1, 3, 4-oxadiazole-2-yl)-H-imidazo [1,2, a] pyridine derivatives 5a-i and 6a-d. Most of the newly synthesized compounds exhibited promising in vitro antibacterial and antifungal activity. Among the investigated compounds 5b, 5c, 5d, 5e, 5f, 5g, 5h, 5i, 6a, 6c and 6d were more potent towards bacterial strains and 5d, 5g, 5h and 6c were effective against fungal strains. Remaining compounds also showed moderate to weak antimicrobial activities.

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