Synthesis and antimicrobial activity studies of certain pyrimidinyl oxadiazolo azetidinones

Sonia George*, R Sabitha, V Govindhammal & T K Ravi
Department of Pharmaceutical Chemistry, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences Coimbatore 641 044, India
E-mail: soniashenjeev@gmail.com

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Aryl substituted pyrimidinyl oxadiazolo azetidinones have been synthesized from ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate 1 which on reaction with semicarbazide hydrochloride in ethanol yielded 2-[(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-yl)carbonyl]hydrazinecarboxamide 2. Compound 2, on reaction with concentrated sulphuric acid, underwent cyclization to yield 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one 3. Further, compound 3, on treatment with appropriate aldehydes, in ethanol gave 6-methyl-4-phenyl-5-[(5-[(1E)-arylmethyldene]amino)-1,3,4-oxadiazol-2-yl]-3,4-dihydropyrimidin-2(1H)-ones 4a-e. Finally, the title compounds 5-[5-(3-chloro-2-oxo-4-arylamidinyl)-1,3,4-oxadiazol-2-yl]-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-ones 5a-e have been obtained by ring closure of the compounds 4a-e with monochloroacetyl chloride in dioxane in the presence of triethylamine. The structures of the novel compounds were assigned based on IR, 1H NMR, mass spectral data and elemental analysis data. All the compounds have been screened for their antimicrobial activity using bacterial and fungal strains.

Keywords: Pyrimidinyl oxadiazoles, azetidinones, antibacterial activity, antifungal activity

Pyrimidines through their varied biological activity stand one among the significant scaffolds which requires further exploration. They are reported to possess activities like antimicrobial, antioxidant, anticancer, antimalarial, etc. Oxadiazoles are promising moieties possessing antimicrobial, tuberculostatic, anticancer, anti-inflammatory activity, etc. Azetidinones with their well known biological importance especially as antimicrobial, antitubercular, antiviral, anticonvulsant etc gaining much importance. The present synthetic work is planned by keeping in view of the biological potential of these three moieties, and the effect of the incorporation of these moieties is probed in the study.

The starting compound 1 was synthesized by following a literature protocol. The homogeneity of the compounds was ascertained by TLC and the structures were confirmed by spectral characterization.

Results and Discussion

Aryl substituted pyrimidinyl oxadiazolo azetidinones have been synthesized from ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate 1 which on reaction with semicarbazide hydrochloride in ethanol yielded 2-[(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-yl)carbonyl]hydrazinecarboxamide 2. Compound 2, on reaction with concentrated sulphuric acid, underwent cyclization to yield 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one 3. Further, compound 3, on treatment with appropriate aldehydes, in ethanol gave 6-methyl-4-phenyl-5-[(5-[(1E)-arylmethyldene]amino)-1,3,4-oxadiazol-2-yl]-3,4-dihydropyrimidin-2(1H)-ones 4a-e. Finally, the title compounds 5-[5-(3-chloro-2-oxo-4-arylamidinyl)-1,3,4-oxadiazol-2-yl]-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-ones 5a-e have been obtained by ring closure of the compounds 4a-e with monochloroacetyl chloride in dioxane in the presence of triethylamine (Scheme 1). The structures of the novel compounds were assigned based on IR, 1H NMR, mass spectral data and elemental analysis data.

Antimicrobial activity

The in vitro antimicrobial studies were performed. The newly synthesized compounds were screened in vitro for their antibacterial activity using the bacterial strains, Micrococcus luteus NCIM 2704, Pseudomonas aeruginosa NCIM 5029, Escherichia coli NCIM 2118 and Bacillus subtilis NCIM 2010 and fungal strains, Asperrigillus niger NCIM 545 and Candida albicans NCIM 3100 by Kirby beaur disc diffusion method and the zone of inhibition was measured. Standard drugs used for bactericidal and antifungal screening were ciprofloxacin and fluconazole respectively at 10 μg/disc. The results of the antimicrobial activity screening is given in Table I.

The antibacterial screening results reveal that compounds 5b, 5d and 5e showed good activity
Scheme I

Table I — Antimicrobial activity of pyrimidinyl oxadiazolo azetidinones 5a-e

<table>
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<th>Compd</th>
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<td>P. aeruginosa</td>
<td>E. coli</td>
<td>B. subtilis</td>
<td>A. niger</td>
<td>C. albicans</td>
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<tr>
<td>5a</td>
<td>20</td>
<td>22</td>
<td>18</td>
<td>19</td>
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<tr>
<td>5b</td>
<td>30</td>
<td>34</td>
<td>21</td>
<td>21</td>
<td>31</td>
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<tr>
<td>5c</td>
<td>18</td>
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<td>34</td>
<td>20</td>
<td>20</td>
<td>32</td>
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<td></td>
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<tr>
<td>5e</td>
<td>30</td>
<td>33</td>
<td>18</td>
<td>18</td>
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<td>40</td>
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<td>37</td>
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<tr>
<td>Fluconazole</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>37</td>
<td>30</td>
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</tbody>
</table>

Ar: H, 3-OCH₃, 3,4,5-OCH₃, 4-F, 2-Cl
against *Micrococcus luteus* and *Pseudomonas aeruginosa*. The other derivatives 5a and 5c were only moderately active. All the derivatives were only moderately active against both the gram negative species.

The anifungal results revealed that the compounds 5b and 5d showed significant activity against *Aspergillus niger* and compounds 5a, 5d and 5e showed good activity against *Candida albicans*. The other derivatives were only moderately active against both the fungal species.

### Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. TLC was accomplished on precoated silica gel plates and iodine vapour was used as visualizing agent. IR spectra were recorded on Jasco FT-IR 410 instrument. $^1$H NMR spectra on FT-NMR Bruker 300 MHz instrument and mass spectra were recorded on Shimadzu LCMS-2010A spectrometer. Elemental analysis was done on Vario EL III C, H, N, O analyzer.

### Experimental procedure for the preparation of 2-[(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)carbonyl]hydrazinecarboxamide, 2

To a solution of compound 1 (19.5 g, 0.075 mol) in ethanol (50 mL), semicarbazide hydrochloride (5.62 g, 0.075 mol) was added and refluxed for 10 hr in the presence of anhydrous sodium hydroxide (5 g). The excess solvent was distilled off under reduced pressure and the resulting solid mass was poured into ice-water, filtered, washed with water (3 × 10 mL), and then recrystallised from absolute ethanol (30 mL). The TLC solvent system used was toluene: methanol (6:4). m.p. 205°C; Yield: 1.73 g, 64%; R$_f$: 0.73. IR (KBr): 3250 (NH), 3089 (Ar-H), 2948 (C-H), 1650 (C=O), 1624 (C=C), 1040 cm$^{-1}$ (N-N). $^1$H NMR (300 MHz, DMSO-d$_6$): δ 2.45 (s, 3H, C-CH$_3$), 3.10 (s, 1H, C-NH-CO), 3.65 (s, 3H, OCH$_3$), 6.25 (s, 2H, NH$_2$), 7.69-7.98 (m, 5H, Ar-H), 8.62 (s, 1H, Ar-H). 9.36 (s, 1H, NHC$_6$H$_5$); MS: m/z 272 (m+1)$^+$. Anal for C$_{13}$H$_{13}$N$_2$O$_2$: Found: C, 54.15; H, 5.67; N, 24.31. Requires: C, 53.97; H, 5.79; N, 24.22%.

### Experimental procedure for the preparation of 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one, 3

A mixture of compound 2 (14.45 g, 0.05 mol) and concentrated sulphuric acid (20 mL) was kept overnight (15 hr) at RT. Ice cold water (300 mL) was added to the reaction mixture and the content was shaken. The reaction mixture was neutralized with liquid ammonia. The solid obtained was washed with water and recrystallised from methanol (25 mL). The TLC solvent system used was toluene: methanol (3:7). m.p. 343°C; Yield: 10.11 g, 70%; R$_f$: 0.70.

IR (KBr): 3355 (NH), 3082 (Ar-H), 2960 (C-H), 1669 (C=N), 1618 (C=C), 1422 (N=N), 1266 cm$^{-1}$ (C-N); $^1$H NMR (300 MHz, DMSO-d$_6$): δ 2.56 (s, 3H, C-CH$_3$), 3.15 (s, 1H, C-NH-CO), 6.30 (s, 1H, Ar-CH), 6.25 (s, 2H, NH$_2$), 7.69-7.98 (m, 5H, Ar-H), 9.36 (s, 1H, NHC$_6$H$_5$); MS: m/z 272 (m+1)$^+$. Anal for C$_{13}$H$_{13}$N$_2$O: Found: C, 54.07; H, 5.67; N, 24.31. Requires: C, 53.97; H, 5.79; N, 24.22%.

### General procedure for the preparation of 6-methyl-4-phenyl-5-[(1E)-arylmethylidene] amino]-1,3,4-oxadiazol-2-yl)-3,4-dihydropyrimidin-2(1H)-one, 4a-e

A mixture of compound 3 (2.71 g, 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in absolute ethanol (25 mL) was refluxed for 4 hr and filtered while hot. On cooling the filtrate, the solid obtained was collected and purified by recrystallisation from ethanol. The TLC solvent system used was toluene: ethyl acetate (4:3:3).

### 4-Methyl-6-phenyl-5-[(1E)-phenylmethylidene] amino]-1,3,4-oxadiazol-2-yl)tetrahydropyrimidin-2(1H)-one, 4a

Solid; m.p. 205°C; Yield: 1.73 g, 64%; R$_f$: 0.73. IR (KBr): 3365 (NH), 3071 (Ar-H), 2996 (C-H), 1675 (C=O), 1624 (C=C), 1040 cm$^{-1}$ (C-O-C); $^1$H NMR (300 MHz, DMSO-d$_6$): δ 2.51 (s, 3H, C-CH$_3$), 3.10 (s, 1H, C-NH-CO), 6.27 (s, 1H, Ar-CH), 7.85-8.14 (m, 5H, Ar-H), 8.50 (m, 4H, NHNHCONH$_2$), 9.42 (s, 1H, NHC$_6$H$_5$); MS: m/z 290 (m+1)$^+$. Anal for C$_{13}$H$_{13}$N$_2$O$_2$: Found: C, 54.15; H, 5.67; N, 24.31. Requires: C, 53.97; H, 5.79; N, 24.22%.

### 5-(5-[(1E)-(3-Methoxyphenyl) methylidene] amino)-1,3,4-oxadiazol-2-yl)4-methyl-6-phenyltetrahydropyrimidin-2(1H)-one, 4b

Solid; m.p. 219°C; Yield: 1.76 g, 65%; R$_f$: 0.71. IR (KBr): 3379 (NH), 3058 (Ar-H), 2983 (C-H), 1667 (C=N), 1647 (CH=CH$_2$), 1623 (C=C), 1055 (N-N), 1035 cm$^{-1}$ (C-O-C); $^1$H NMR (300 MHz, DMSO-d$_6$): δ 2.49 (s, 3H, C-CH$_3$), 3.20 (s, 1H, C-NH-CO), 3.65 (s, 3H, OCH$_3$), 6.34 (s, 1H, Ar-CH), 7.58-7.89 (m, 9H, Ar-H), 9.27 (s, 1H, Ar-H).
5-(5-[(1E)-(3-Methoxyphenyl) methylidene] amino)-1,3,4-oxadiazol-2-yl)-4-methyl-6-phenyltetrahydro-pyrimidin-2(1H)-one, 4c

Solid; m.p. 309°C; Yield: 1.82 g, 66%; Rf: 0.64. IR (KBr): 3382 (NH), 3068 (Ar-H), 2991 (C-H), 1670 cm⁻¹ (C=O); 1H NMR (300 MHz, DMSO-d₆): δ 2.46 (s, 3H, C-CH₃), 3.23 (s, 1H, C-NH-CO), 3.72 (s, 9H, O(CH₃)₃), 6.31 (s, 1H, Ar-CH), 7.52-7.91 (m, 7H, Ar-H), 8.72 (s, 1H, N=CH), 9.54 (s, 1H, N=CH₂); MS: m/z 450 (m+1)⁺. Anal for C₂₃H₂₃N₃O₄: Found: C, 64.78; H, 4.88; N, 17.99%.

5-(5-[(1E)-(4-Fluorophenyl) methylidene] amino)-1,3,4-oxadiazol-2-yl)-4-methyl-6-(5-(5-[(1E)-5-(3-Chloro-2-(3-methoxyphenyl)-4-oxoazetidin-1-yl)-1,3,4-oxadiazol-2-yl]-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one, 5a

Solid; m.p. 310°C; Yield: 2.29 g, 64%; Rf: 0.58. IR (KBr): 3361 (NH), 3070 (Ar-H), 2988 (C-H), 1668 (C=O), 1653 (C=O), 1630 (C=O), 1061 (N-C), 1037 cm⁻¹ (C-O-C); 1H NMR (300 MHz, DMSO-d₆): δ 2.48 (s, 3H, C-CH₃), 3.34 (s, 1H, C-NH-CO), 4.58 (d, 1H, CH-N azetidine), 6.28 (s, 1H, Ar-CH), 7.4-7.88 (m, 10H, Ar-H), 8.03 (d, 1H, CH-Ch azetidine), 9.66 (s, 1H, NHCH₂CH₃); MS: m/z 436 (m+1)⁺. Anal for C₂₃H₁₈ClN₅O₄: Found: C, 60.69; H, 4.14; N, 16.09%.

5-(5-[(1E)-(2-Chlorophenyl) methylidene] amino)-1,3,4-oxadiazol-2-yl)-4-methyl-6-(5-(5-[(1E)-5-(3-Chloro-2-(3,4,5-trimethoxyphenyl)-4-oxoazetidin-1-yl)-1,3,4-oxadiazol-2-yl]-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one, 5b

Solid; m.p. 310°C; Yield: 2.29 g, 59%; Rf: 0.52. IR (KBr): 3361 (NH), 3065 (Ar-H), 2981 (C-H), 1703 (azetidinyl C=O), 1666 (C=O), 1631 (C=O), 1058 (N-C), 1040 (C-O-C), 777 cm⁻¹ (azetidinyl CH-Cl); 1H NMR (300 MHz, DMSO-d₆): δ 2.51 (s, 3H, C-CH₃), 3.30 (s, 1H, C-NH-CO), 4.58 (d, 1H, CH-N azetidine), 6.28 (s, 1H, Ar-CH), 7.48-7.88 (m, 10H, Ar-H), 8.03 (d, 1H, CH-Ch azetidine), 9.66 (s, 1H, NHCH₂CH₃); MS: m/z 466 (m+1)⁺. Anal for C₂₃H₁₈ClN₅O₄: Found: C, 60.69; H, 4.14; N, 15.21. Requires: C, 59.35; H, 4.30; N, 15.05%.

5-(5-[(1E)-(3-Chloro-2-(3-thienyl)-4-oxoazetidin-1-yl)-1,3,4-oxadiazol-2-yl]-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one, 5c

Solid; m.p. 210°C; Yield: 2.78 g, 62%; Rf: 0.54. IR (KBr): 3366 (NH), 3082 (Ar-H), 2963 (C-H), 1710 (azetidinyl C=O), 1670 (C=N), 1621 (C=O), 1064 (N-C), 1042 (C-O-C), 780 cm⁻¹ (azetidinyl CH-Cl); 1H NMR (300 MHz, DMSO-d₆): δ 2.56 (s, 3H, C-CH₃), 3.33 (s, 1H, C-NH-CO), 3.77 (s, 9H, O(CH₃)₃), 4.65 (d, 1H, CH-N azetidine), 6.36 (s, 1H, Ar-CH), 7.63-7.92 (m, 7H, Ar-H), 8.05 (d, 1H, CH-Ch azetidine), 9.67 (s, 1H, NHCH₂CH₃); MS: m/z 526 (m+1)⁺. Anal for C₂₃H₁₈ClN₅O₄: Found: C, 60.80; H, 4.27; N, 15.21. Requires: C, 59.35; H, 4.30; N, 15.05%.

General procedure for the preparation of 5-[5-(3-Chloro-2-(3,4,5-trimethoxyphenyl)-4-oxoazetidin-1-yl)-1,3,4-oxadiazol-2-yl]-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one, 5a-e

Monochloroacetyl chloride (1.11 g, 0.01 mol) was added dropwise to a mixture of Schiff base 4a-e (0.01 mol) and triethylamine (1.01 g, 0.01 mol) in dioxane (25 mL). The reaction mixture was stirred for 8 hr and left at RT for 3 days. The contents were poured onto crushed ice, filtered and washed with water. The isolated product was recrystallised from dioxane: methanol (3:1) (10 mL). The TLC solvent system used was benzene: chloroform: methanol (6:2:2).
for $C_2H_2ClN_2O_6$ Found: C, 57.28; H, 4.67; N, 13.46. Requires: C, 57.14; H, 4.57; N, 13.33%.

5-{5-[3-Chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-y1]-1,3,4-oxadiazol-2-yl}-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one, 5d
Solid; m.p. 198°C; Yield: 2.37 g, 63%; Rf: 0.60. IR (KBr): 3371 (NH), 3078 (Ar-H), 2959 (C-H), 1708 (azetidinyl C=O), 1656 (C=N), 1638 (C=C), 1055 (N-N), 1048 (C-O-C), 781 cm$^{-1}$ (azetidinyl CH-Cl); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 2.61 (s, 3H, C-CH$_3$), 3.35 (s, 1H, C-NH-CO), 4.64 (d, 1H, CH-N azetidine), 6.40 (s, 1H, Ar-CH), 7.51-7.86 (m, 9H, Ar-H), 8.10 (d, 1H, CH-Cl azetidine), 9.71 (s, 1H, NH$_2$CHC$_6$H$_5$); MS: m/z 453 (m$^+$). Anal for $C_{22}H_{17}ClFN_5O_3$ Found: C, 58.39; H, 3.91; N, 15.60. Requires: C, 58.28; H, 3.75; N, 14.89%.

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Reference