



SYNTHESIS AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF SOME 5- [7-ARYL-6-PHENYL-5-THIOXO 6,7 – DIHYDRO - 5H-[1,3,4] OXADIAZOLO [3,2, -a] [1,3,5] TRIAZIN-2-YL)-6-METHYL-4-PHENEYL-3,4-DIHYDRO PYRIMIDIN-2(1H) - ONE DERIVATIVES

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ABSTRACT

Urea, benzaldehyde and ethyl acetoacetate were condensed by Biginelli reaction to form ethyl 6-methyl -2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1**), which was further made to react with semicarbazide hydrochloride to form 2-[(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl) carbonyl] hydrazine carboxamide (**2**). This product on reaction with concentrated sulphuric acid and further neutralization with ammonia gave 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (**3**). The resultant amino oxadiazolyl pyrimidinone is condensed with different aromatic aldehydes to obtain corresponding Schiff bases **4(a-j)**. These products on reaction with monochloroacetyl chloride and triethylamine gave 5-[5-(3-chloro-2-oxo-4-arylazetid-1-yl)-1,3,4-oxadiazol-2-yl]-2(1H)-one **5(a-j)**. All these final compounds were characterized on the basis of spectral data and screened for their antioxidant activity.

Key words: Pyrimidinyl oxadiazolyl amines, Pyrimidinyl oxadiazolyl azetidinones, Antioxidant activity.

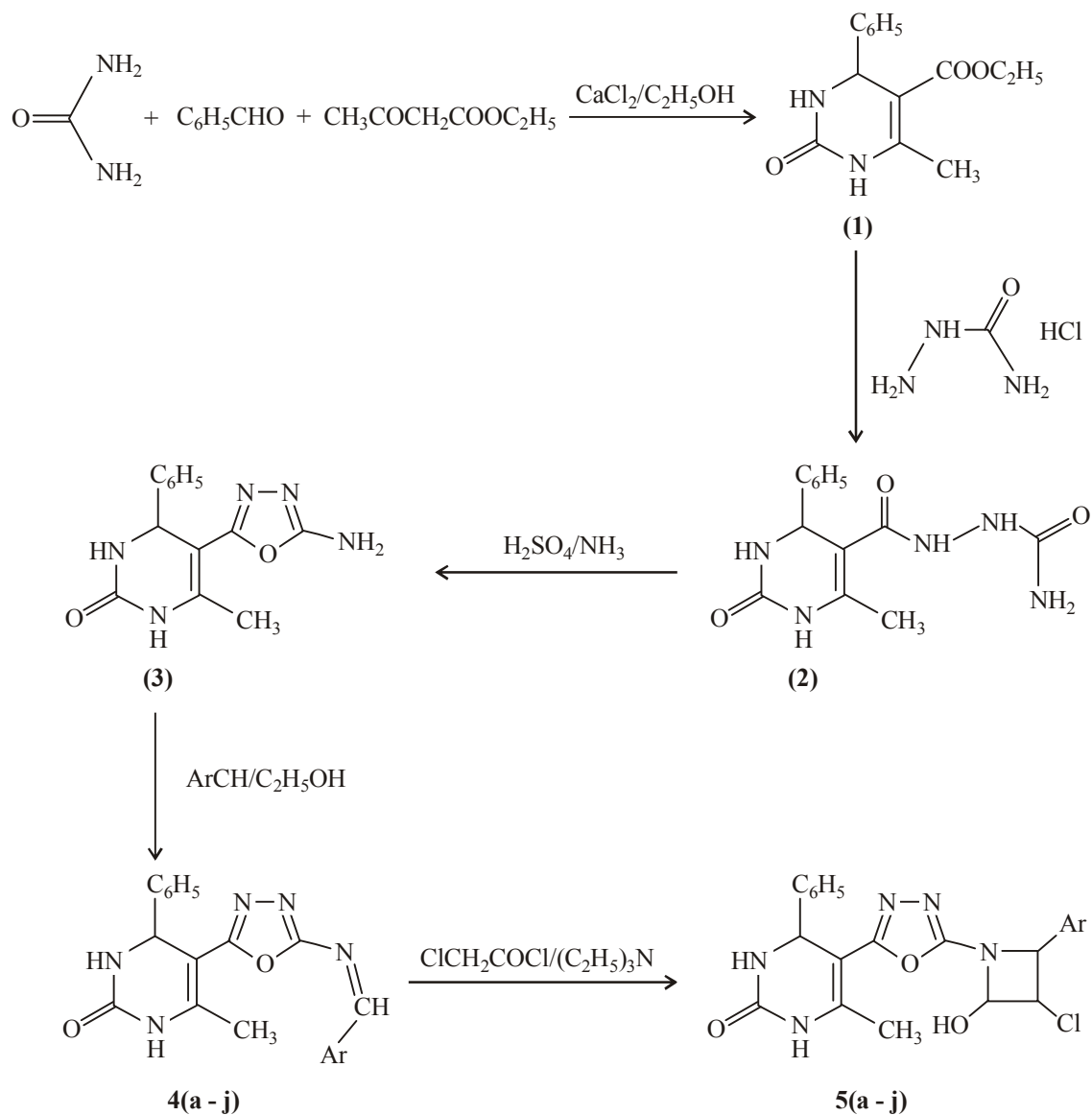
INTRODUCTION

Pyrimidines are well known for their antimicrobial, antioxidant, anticancer, anti-malarial and anticonvulsant activity.¹⁻⁵ Oxadiazoles are also well known for their antimicrobial, anticancer, anti-inflammatory, analgesic and anticonvulsant activity⁶⁻¹⁰. Azetidinones are well known for their antimicrobial, anti tubercular and antiviral activity¹¹⁻¹³. In this work, an effort has been taken to incorporate the pyrimidine, oxadiazole and

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azetidinone nucleus to form different pyrimidinyl oxadiazolyl azetidinones. The synthetic pathway is depicted in scheme 1.

Scheme 1



Ar = (a) $-C_6H_5$, (b) $4-(OCH_3)-C_6H_4$, (c) $3-(OCH_3)-C_6H_4$, (d) $3-(OCH_3)-4-(OH)-C_6H_3$ (e) $Cl-C_6H_4$, (f) $3-NO_2-C_6H_4$, (g) $3,4,5-(OCH_3)-C_6H_2$, (h) $4-N,N-(CH_3)-C_6H_2$, (i) $4-F-C_6H_2$ and (j) $2-Cl-C_6H_2$

EXPERIMENTAL

Material and methods

Melting points were determined by using MP-II, Veego Instruments Corporation (India) and were uncorrected. Purity of the products were tested by TLC using glass plates coated with silica gel G and benzene : chloroform : methanol (6 : 2 : 2) as the developing solvent. IR spectra were recorded using KBr on a JASCO FT-IR -410 spectrophotometer (Japan).

¹H NMR spectra were recorded in CDCl₃ on FT-NMR Bruker (Germany) using tetramethylsilane as an internal standard. Mass spectra were recorded on a Shimadzu LCMS-2010A mass spectrometer (70 eV) (Japan).

Preparation of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (1)¹⁴

A solution of ethyl acetoacetate (7.6 mmoles), benzaldehyde (7.6 mmoles) and urea (9.2 mmoles) in ethanol was refluxed in the presence of calcium chloride (0.168 g) for 2 hours (TLC). The reaction mixture was then poured onto crushed ice and solid product separated was filtered and recrystallised from ethanol. m.p 200°C; Yield 75%.

Preparation of 2-[(6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidin-5-yl) carbonyl] hydrazine carboxamide (2)

To a solution of compound (1) (0.075 mole) in ethanol, semicarbazide hydrochloride (0.075 mole) was added and refluxed for about 10 hours in the presence of anhydrous sodium hydroxide. The excess solvent was distilled off under reduced pressure and resulting solid mass poured into ice-water, filtered, washed several times with water and then recrystallised from absolute ethanol. m.p 205°C; Yield 64%.

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) (0.05 mole) and concentrated sulphuric acid (20 mL) was kept overnight at room temperature and then 300 mL ice cold water was added into the reaction mixture and the contents were shaken. Then reaction mixture was neutralized with liquid ammonia. The solid obtained was washed with water and recrystallized with methanol. m.p 208°C; Yield 66%.

Preparation of 6-methyl-4-phenyl-5-(5-[(1E)-aryl methylidene] amino)-1, 3, 4-oxadiazol-2-yl)-3,4-dihydropyrimidin-2(1H)-one 4(a-j)

A mixture of compound (3) (0.01 mole) and the appropriate aromatic aldehyde (0.01 mole) in absolute ethanol (25 mL) was refluxed for 4 hours and filtered while hot. On cooling the filtrate, the solid obtained was filtered and purified by recrystallisation from ethanol.

Preparation of 5-[5-(3-chloro-2-oxo-4-arylazetid-1-yl)-1, 3, 4-oxadiazol-2-yl]-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one 5(a-j)

Monochloroacetyl chloride (0.01 mole) was added dropwise to a mixture of (0.01 mole) 4(a-j) and triethylamine (0.1 mL) in dioxane (25 mL). The reaction mixture was stirred for 8 hours and left at room temperature 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).

Antioxidant activity¹⁵

Free radical scavenging activity of the test compounds 5(a-j) were performed by diphenylpicryl hydrazyl (DPPH) assay method. The drug solutions were prepared in 0.1mM, 0.2 mM and 0.5 mM concentrations. 1.5 mL of each solution was taken in separate test tube and 1.5 mL of 0.2 mM DPPH solution was added to it and allowed to react at room temperature. After 30 minutes, the absorbance values were measured at 517 nm and converted into the percentage antioxidant activity (AA%).

It was calculated by the formula-

$$AA\% = [A_b + A_s - A_m / A_b] \times 100$$

where, A_b = Absorbance of 1.5 mL DPPH + 1.5 mL methanol, A_m = Absorbance of 1.5 mL DPPH + 1.5 mL drug solution and A_s = Absorbance of 1.5 mL drug solution + 1.5 mL methanol. Methanol was used as a solvent for the above studies and ascorbic acid was used as the standard. The trials were done in triplicate. The results are presented in Table 2.

RESULTS AND DISCUSSION

The reaction of 6-methyl-4-phenyl-5-(5-[(1E)-aryl methylidene] amino)-1,3,4-oxadiazol-2-yl)-3,4-dihydropyrimidin-2(1H) – one 4(a-j) with monochloroacetyl chloride and triethyl amine gave the target compounds 5-[5-(3-chloro-2-oxo-4-arylazetid-1-yl)-1,3,4-oxadiazol-2-yl]-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one 5(a-j).

Table 1: Spectral data of compounds 4(a-j) and 5(a-j)

Compound	IR (cm⁻¹), ¹H NMR (δ) and Mass (m/z) spectral data
4a	3474, 2932, 1726, 1645, 1596, 1291, 1092; 7.24-7.38 (m,11H,Ar-H), 5.8 (s,1H,N=CH), 5.12 (s,1H,N ₁ -H), 5.38-5.42 (d,1H,N ₃ -H), 1.26 (s,3H,C ₆ -CH ₃); 359
4b	3470, 2935, 1720, 1646, 1582, 1280,1090; 7.21-7.72 (m,10H,Ar-H), 5.76 (s,1H,N=CH), 5.15 (s,1H,N ₁ -H), 5.32-5.41 (d,1H,N ₃ -H), 3.7 (s,3H,C -OCH ₃),1.32(s,3H,C ₆ -CH ₃); 389
4c	3486, 2948, 1746, 1658, 1596, 1285,1088; 7.38-7.76 (m,10H,Ar-H), 5.82 (s,1H,N=CH), 5.42- 5.62 (d,1H,N ₃ -H), 5.20 (s,1H,N ₁ -H), 3.8 (s,3H,C -OCH ₃), 1.26 (s,3H,C ₆ -CH ₃); 389
4d	3495, 2916, 1718, 1662, 1585, 1276,1078; 7.78(s,1H,OH), 7.42-7.68 (m,9H,Ar-H), 5.72 (s,1H,N=CH), 5.25 (s,1H,N ₁ -H), 5.38-5.56 (d,1H,N ₃ -H), 1.19 (s,3H,C ₆ -CH ₃), 3.7 (s,3H,C -OCH ₃); 405
4e	3492, 2962, 1715, 1655, 1578, 1288, 1065; 7 .49-7.8 (m,10H,Ar-H), 5.78(s,1H,N=CH), 5.26 (s,1H,N ₁ -H), 5.46-5.56 (d,1H,N ₃ -H), 1.21 (s,3H,C ₆ -CH ₃); 394
4f	3487, 2948, 1734, 1662, 1588, 1291, 1072; 7.27-7.72 (m,10H,Ar-H), 5.73 (s,1H,N=CH), 5.30 (s,1H,N ₁ -H), 5.36-5.56(d,1H,N ₃ -H), 1.28 (s,3H,C ₆ -CH ₃); 404
4g	3489, 2972, 1742, 1670, 1566, 1271, 1083; 7.24-7.70 (m,8H,Ar-H), 5.81 (s,1H,N=CH), 5.19 (s,1H,N ₁ -H), 5.32-5.45 (d,1H,N ₃ -H), 3.8 (s,6H,C-OCH ₃ ,C-OCH ₃), 3.6 (s,3H,C- OCH ₃), 1.25 (s,3H,C ₆ -CH ₃); 449
4h	3458, 2938, 1720, 1648, 1570, 1277, 1075; 7.27-7.44 (m,10H,Ar-H), 5.75 (s,1H,N=CH), 5.28 (s,1H,N ₁ -H), 5.4 -5.56 (d,1H,N ₃ -H), 1.6 (s, 6H,C -N(CH ₃) ₂), 1.25 (s,3H,C ₆ -CH ₃); 402
4i	3499, 2962, 1755, 1678, 1538, 1273, 1068; 7.30-7.52 (m,10H,Ar-H),5.83(s,1H,N=CH),5.15 (s,1H,N ₁ -H), 5.36-5.44 (d,1H,N ₃ -H),1.28 (s,3H,C ₆ -CH ₃); 377
4j	3491, 2956, 1748, 1661, 1573, 1287, 1084; 7.28-7.49 (m,10H,Ar-H), 5.82(s,1H,N=CH), 5.10 (s,1H,N ₁ -H), 5.35-5.48 (d,1H,N ₃ -H), 3.8 (s, 3H, OCH ₃),1.24 (s,3H,C ₆ -CH ₃); 394

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Compound	IR (cm ⁻¹), ¹ H NMR (δ) and Mass (m/z) spectral data
5a	3247, 3113, 2932, 1726, 1092, 780; 7.8 (d,1H, N ₃ -H), 7.2-7.4 (m,12H,Ar-H), 5.140 (s,1H, N ₁ -H), 2.5 (s,1H,CH-Cl), 2.084 (s,3H,C ₆ -CH ₃); 435
5b	3241, 3119, 2931, 1721, 1093, 772; 7.83 (d, 1H, N ₃ -H), 7.3-7.6 (m,11H,Ar-H), 5.133 (s,1H, N ₁ -H), 3.9 (s,3H,C-OCH ₃) 2.44 (s,1H,CH-Cl), 2.077 (s,3H,C ₆ -CH ₃); 466
5c	3255, 3125, 2919, 1728, 1086, 790; 7.82 (d, 1H, N ₃ -H), 7.18-7.44 (m,11H,Ar-H), 5.143 (s,1H, N ₁ -H), 3.8 (s,3H,C-OCH ₃) 2.48 (s,1H,CH-Cl), 2.1 (s,3H,C ₆ -CH ₃); 466
5d	3245, 3120, 2928, 1730, 1090, 782; 7.82 (d, 1H, N ₃ -H), 7.79 (s,1H,OH), 7.20-7.44 (m,10H,Ar-H), 5.139 (s,1H, N ₁ -H), 3.8 (s,3H,C-OCH ₃), 2.48 (s,1H,CH-Cl), 2.1 (s,3H,C ₆ -CH ₃); 482
5e	3241, 3117, 2938, 1720, 1095, 771; 7.77 (d,1H, N ₃ -H), 7.4-7.6 (m,11H,Ar-H), 5.139 (s,1H, N ₁ -H), 2.54 (s,1H,CH-Cl), 2.078 (s,3H,C ₆ -CH ₃); 470
5f	3250, 3123, 2929, 1731, 1089, 781; 7.82 (d,1H, N ₃ -H), 7.27-7.39 (m,11H,Ar-H), 5.142 (s,1H, N ₁ -H), 2.58 (s,1H,CH-Cl), 2.099 (s,3H,C ₆ -CH ₃); 481
5g	3250, 3110, 2940, 1725, 1094, 781; 7.80 (d,1H, N ₃ -H), 7.47-7.6 (m,9H,Ar-H), 5.141 (s,1H, N ₁ -H), 3.9 (s,6H,C-OCH ₃), 3.8 (s,3H,C-OCH ₃) 2.54 (s,1H,CH-Cl), 2.078 (s,3H,C ₆ -CH ₃); 526
5h	3245, 3107, 2933, 1728, 1088, 788; 7.81 (d,1H, N ₃ -H), 7.43-7.6 (m,11H,Ar-H), 5.138 (s,1H, N ₁ -H), 2.57 (s,1H,CH-Cl), 2.080 (s,3H,C ₆ -CH ₃), 1.70 (s,6H,C-N(CH ₃) ₂); 479
5i	3250, 3114, 2938, 1720, 1092, 781; 7.79 (d, 1H, N ₃ -H), 7.32-7.58 (m,11H,Ar-H), 5.140 (s,1H, N ₁ -H), 2.42 (s,1H,CH-Cl), 2.084 (s,3H,C ₆ -CH ₃); 454
5j	3244, 3116, 2933, 1728, 1095, 783; 7.81 (d, 1H, N ₃ -H), 7.3-7.6 (m,11H,Ar-H), 5.145 (s,1H, N ₁ -H), 2.52 (s,1H,CH-Cl), 2.083 (s,3H,C ₆ -CH ₃); 470

In the present study, the intermediate 6-methyl-4-phenyl-5-(-[1E]-aryl methylidene] amino)-1,3,4-oxadiazol-2-yl)-3,4-dihydropyrimidin-2(1H)-ones (**4a**) formed from the reaction of 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (**3**) with the appropriate aromatic aldehyde showed IR bands at 3474, 2932, 1726, 1645, 1596, 1291 and 1092 cm^{-1} , respectively for N-H, C-H aliphatic, C=O aromatic, C=C aromatic, C=N, C-N, and C-O-C. Its ^1H NMR spectra (Table 2) showed multiplets between 7.24 – 7.38 indicating the presence of eleven aromatic protons. A singlet at 5.8 accounted for the N=CH proton. A doublet at 5.38-5.42 represents the proton attached to nitrogen at third position of pyrimidine and that at 5.12 accounted for the proton attached to nitrogen at first position of pyrimidine. A singlet at 1.23 ppm accounted for methyl proton on the pyrimidine nucleus. The mass spectrum showed the molecular ion at m/z 359. 6-Methyl-4-phenyl-5-(5-{5-(1E)-aryl methylidene] amino}-1,3,4-oxadiazol-2-yl)-3,4-dihydropyrimidin-2(1H)-one (**4a**) on refluxing with monochloroacetyl chloride and triethyl amine afforded 5-[5-(5-chloro-2-oxo-4-phenylazetid-1-yl)-1,3,4-oxadiazol-2-yl]-6-methyl-4-phenyl 3,4-dihydropyrimidin-2(1H)-one (**5a**). Its IR spectrum (Table 2) showed bands at 3247, 3113, 2932, 1726, 1092 and 780 cm^{-1} , respectively for N-H, C-H aromatic, C-H aliphatic, C=O aromatic, C-O-C and C-H aromatic bending. NMR spectra (Table 2) showed a doublet at δ 7.8 corresponding to $\text{N}_3\text{-H}$ of the pyrimidine nucleus. Multiplets between 7.2-7.4 showed the presence of twelve aromatic protons. A singlet at 5.14 represents the proton attached to nitrogen at first position of pyrimidine and that at 2.5 ppm accounted for the CH-Cl proton. A singlet at 2.084 accounted for methyl proton on the pyrimidine nucleus. The mass spectrum showed the molecular ion at m/z 435. Major fragmentation peaks appeared at 255, 281, 109 and 230. From these facts, it was concluded that compounds (**5a-j**) were formed successfully. Antioxidant activities of all the newly prepared compounds are shown in Table 2.

Table 2: Antioxidant activity of the compounds 4(a-j) and 5(a-j)

Compound	% Antioxidant activity		
	0.5 mM	0.2 mM	0.1 mM
5a	71.52	65.89	58.71
5b	15.16	12.92	9.82
5c	13.64	11.77	9.40
5d	13.74	11.74	8.12
5e	4.16	2.93	1.48

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Compound	% Antioxidant activity		
	0.5 mM	0.2 mM	0.1 mM
5f	14.45	3.83	1.85
5g	32.80	14.06	4.45
5h	23.33	13.54	12.09
5i	75.30	62.28	55.47
5j	15.70	10.28	8.43
Ascorbic acid	-	-	96.69

The antioxidant studies revealed that compounds (**5a**) and (**5i**) having unsubstituted phenyl group and fluorophenyl group showed good free radical scavenging activity, when compared to the standard, ascorbic acid. None of the other compounds showed antioxidant activity.

CONCLUSION

On the basis of these results, it can be concluded that the incorporation of the pyrimidine, 1,3,4-oxadiazole and azetidinone nucleus proved to be effective in the case of antioxidant studies as the compounds (**5a**) and (**5i**) showed good free radical scavenging activity. Studies on other activities will be performed further.

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