Synthesis, Characterization and *In Vitro* Pharmacological Screening of 3, 4-Dihydropyrimidin-2-one Derivatives

Dey Biplab Kumar, Alam Faruk*, Kemisetti Durgaprasad, **Yakin Josef and Islam Mohidul**
Faculty of Pharmaceutical Science, Assam Down Town University, Panikhaiti, Gandhinagar, Guwahati, Assam, 781026, INDIA
*****faruk_2007a@rediffmail.com

Abstract

Reaction of ethyl-6-methyl–2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidin-5-carboxylates (1) with hydrazine hydrate yielded 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidin-5-carbohydrazide (2). These products on reaction with cyanogen bromide gave 5-(5-amino 1, 3, 4-oxadiazol-2-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2 (1H)-ones (3). The resultant amino-oxadiazolylpyrimidinones were condensed with substituted aromatic aldehyde to obtain various 5-(5-{[-(4-substituted phenyl) methylidene]-amino}-1, 3, 4-oxadiazol-2-yl)-6-methyl-4-phenyl-3, 4-dihydro-pyrimidin-2-one (4a-f). These products were characterized by IR, 1H NMR, mass spectra and elemental analysis.

The synthesized compounds (4a-f) were evaluated for their antimicrobial activity against two gram positive and two gram negative bacteria along with two fungal species /yeast strains. All the synthesized compounds showed good antimicrobial activity. In addition, the newly synthesized compounds were screened for their antioxidant properties. The results showed that the compounds 5-{5-[(4-chlorobenzylidene)-amino]-1, 3, 4- oxadiazol – 2 –yl } -6 – methyl – 4 - phenyl-3, 4 dihydropyrimidin -2 (1H) -one (4b) and 5-{5-[(4-hydroxybenzylidene)-amino]-1,3,4-oxadiazol-2-yl}-6-methyl-4-phenyl-3,4-dihydro pyrimidin- 2 (1H)-one (4d) revealed the highest antimicrobial and antioxidant activity throughout this work.

Keywords: Synthesis, antibacterial, antifungal, antioxidant, DPP-4, α-amylase.

Introduction

Nowadays the multidrug resistant bacteria create a need for the synthesis of new antimicrobial agents. Fused pyrimidines continue to draw significant attention due to their boundless practical usefulness, primarily because of very wide spectrum of biological activities. This is evident in particular from publications of the chemistry of systems where the pyrimidine ring is fused to numerous heterocycles such as quinazolines, purines, pyridopyrimidines, tri-azolopyrimidines, pyrimidoazepines, pyrazolopyrimidines, furopyrimidines. Pyrimidines and pyralopyrimidines are well known for their anticancer[23], antimicrobial[15], antioxidant[12], antidiabetic[16] and antiviral activities.

Recently, there has been vast interest in compounds which contain the oxadiazole scaffold due to their unique chemical structure and their broad spectrum of different biological properties. Oxadiazoles are the heterocyclic compounds which contain one oxygen atom and two nitrogen atoms in a five membered ring[7,20] possessing different useful biological activities. Oxadiazole is considered to be product on replacement of two methane (–CH=) groups by two pyridine type nitrogen atoms (–N=) on furan ring[5]. The 1, 3, 4-oxadiazole derivatives were found to exhibit various biological activities such as antimicrobial[18], anti HIV[8], antitubercular[13], antimalarial[10], anti-inflammatory[3], anticonvulsant[24] and antitumor[14]. The 2, 5-disubstituted-1, 3, 4-oxadiazole derivatives are known for various pharmacological activities such as antibacterial[1] and anticonvulsant.[11]

A current rational approach of drug design involves connecting two molecules having individual intrinsic activity into a lone hybrid molecule with improved efficacy and minimum toxicity[19]. This encouraged us to synthesize 1, 3, 4-oxdiazole groups incorporated with Schiff's bases evaluate their biological activities. In the current study, a series of new fused pyrimidine-oxadiazoles derivatives (4a-f) have been synthesized and their biological activities are determined.

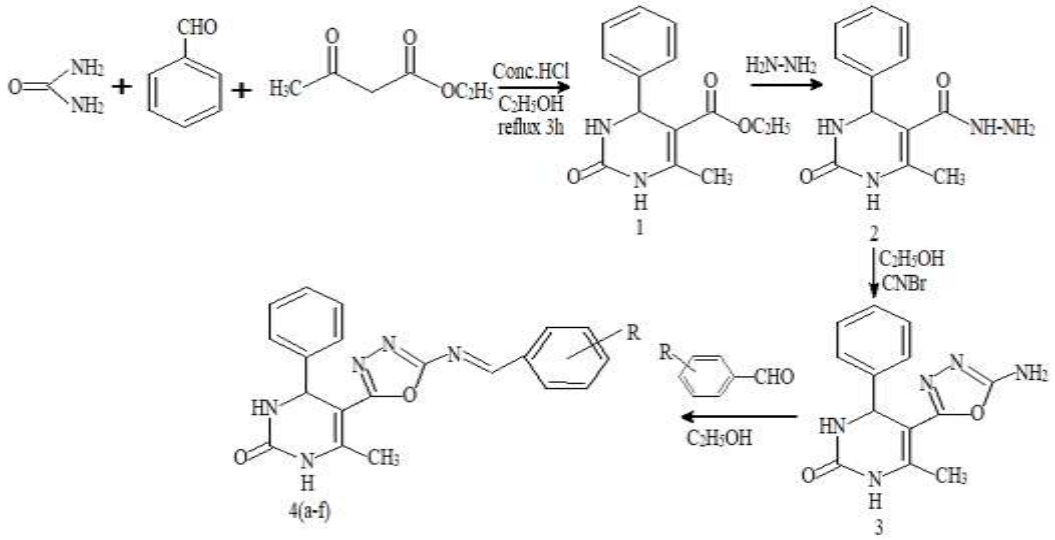
Material and Methods

All the analytical reagent grade (anhydrous) chemicals and solvents were used in this work and purchased from Sigma-Aldrich and S.D. Fine. By using an electrothermal apparatus in an open capillary tube, melting points were measured. Microanalysis was done on a Perkin Elmer model 240 CHN analyzer (USA).

The ^{1}H-NMR were recorded on FT-NMR Brucker (Germany) using TMS as internal reference in $CDCl_3$. Infrared spectra were recorded from KBr discs on Perkin Elmer FT-IR spectrophotometer.

Spectra of Mass were recorded on a Shimadzu QP 5000 mass spectrometer (70 eV) (Japan). Products purity was tested by using TLC glass plates which were coated with silica gel G and ethanol/chloroform/acetone/n-hexane (1:1:4:2) as the developing solvent.

Experimental: All the new compounds were synthesized by following the scheme of reactions as shown in scheme I. General procedures of all steps were involved in the synthesis of substituted pyrimidine-oxadiazoles derivatives.



R= C_6H_5, 2-Cl-C_6H_4 , 2-OH-C_6H_4 , 4-OH-C_6H_4 , 4-NO_2-C_6H_4 , 4-F-C_6H_4

Scheme 1: Synthetic route of the target compounds 4a-4f

Synthesis of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydro pyrimidin-5-carboxylates (1): Urea (0.5mole), ethylacetoacetate (0.75mole) and benzaldehyde (0.75mole) were mixed in ethanol (25 ml). To the mixture, a catalytic volume of concentrated HCl was added, after that the mixture was refluxed for three hours. The contents of mixture were then kept in refrigerator for overnight and the solid products separated out were filtered off. The filtrate was then further refluxed on a water bath for 2 hours. On cooling, solid products were separated out and products were filtered and recrystallized from ethanol [m.p. 212-214 ^{0}C].[17]

Synthesis of 6-methyl-2-oxo--4-phenyl-1, 2, 3, 4-tetrahydropyrimidin-5-carbohydrazides (2)[17]: To 0.1 mole of 1 in 20ml of ethanol, (0.1mole) hydrazine hydrate was added followed by a catalytic volume of concentrated H_2SO_4 (4 drops). The mixture was then refluxed for two hours. Excess amount of solvent was removed and on cooling, a solid product was formed and that was crystallized from ethanol [m.p. 198-199 ^{0}C].

Synthesis of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-6-methyl-4-phenyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidin-2-(1H)-ones (3): To 0.01 ml of 2 in absolute ethanol (25 ml), an aqueous solution of sodium bicarbonate (2 gm in 5 ml water) was added and stirred for a few minutes at room temperature. Cyanogen bromide (0.01 mole, 1.05 gm) was added and stirring was being continued for 36 hours. Concentration of the reaction mixture to one fourth of its volume left a residue, which was poured over crushed ice[21].The separated solid product was then filtered and dried. After that crystallization was done from ethanol when product 3 was obtained. The same procedure was repeated again for the other compounds of the series.

Synthesis of 5-{5-[(substitutedbenzylidene)-amino]-1, 3,4-oxadiazol-2-yl}-6-methyl-4-phenyl-3, 4-dihydro-pyrimidin-2(1H)-one [4(a-f)]: In the presence of a catalytic volume of glacial acetic acid, compound 3 (0.01 mol) and substituted (0.01 mol) aryl aldehyde were refluxed in methanol for 30 mins and cooled. The solid product separated was filtered off and crystallized[21]. The other derivatives were prepared in the same manner.

5-[5-(benzylideneamino)-1, 3, 4-oxadiazol-2-yl]-6-methyl -4-phenyl-3,4-dihydropyrimidin-2(1H) -one(4a): M. F: $C_{20}H_{17}N_5O_2$; M. Wt.: 359; Yield: 89%; M.P: 339–341^{0}C; R_f: 0.67; Elemental analysis (calcd./found): C(66.84/66.01), H(4.77/4.60), N(19.49/ 19.46), O(8.90%); IR (KBr cm^{-1}): 1250, 1345 (C-N str.), 1740 (C=O str.), 3142 (NH str.), 1456, 1612(C=C Ar str.); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.48–7.62 (m, 5H, Ar-H), 7.29–7.82 (m, 5H, Ar-H), 5.1 (s, 1H, N1-H), 5.31-5.34 (d, 1H, N3-H), 1.26 (s, 3H, C6-CH_3); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.48-7.62 (m, 10H, Ar-H), 7.8-8.20 (m, 8H, Ar-CH), 5.1 (s, 1H, N1-H), 5.31–5.34 (d, 1H, N3-H), 1.26 (s, 3H, C6-CH_3); Mass (*m/z*): 359(M)$^+$

5-{5-[(4-chlorobenzylidene)-amino]-1, 3, 4- oxadiazol – 2 –yl} -6 – methyl – 4 - phenyl-3, 4 dihydropyrimidin -2 (1H) -one (4b): M.F: $C_{20}H_{16}ClN_5O_2$; M. Wt.: 393.8; Yield: 91%; M.P: 301–303^{0}C; R_f: 0.58 ; Elemental analysis (calcd./found): C(60.99/61.01), H(4.09/4.10), Cl(9.00/8.98), N(17.78, 16.99), O(8.13/8.06); IR (KBr cm^{-1}): 3420 (N-H str.), 1465 (C-N str.), 1550 (C=C str.), 762(C-Cl str.), 1720 (C=O str.), 1450 (Ar.C-H str.), 1670 (Ar.C-C str.); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.65-7.71 (m, 10H, Ar-H), 6.90-8.06 (m, 8H, Ar-CH), 8.46 (s, 1H, CH=N), 6.70-6.78 (d, 1H, N3-H), 4.80 (s, 1H, N1-H), 1.24 (s, 3H, C6-CH_3);Mass (*m/z*): 394(M+1) $^+$

5-{5-[(2-hydroxybenzylidene)-amino]-1, 3, 4-oxadiazol-2 -yl}-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (4c): M.F: $C_{20}H_{17}N_5O_3$; M.Wt.: 375.3; Yield: 79%; M.P: 308–310^{0}C; R_f: 0.72; Elemental analysis (calcd./found): C(63.99/64.00), H(4.56/4.67), N(18.66/18.43), O(12.79/

12.68); IR (KBr cm^{-1}): 3455 (-OH str.), 3550 (N-H str.), 1450 (C-N str.), 1500 (C=C str.), 1754 (C=O str.), 1550 (Ar.C-H str.), 1690 (Ar.C-C str.); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 6.80-8.20 (m, 8H, Ar-CH), 9.41 (s,1H, OH), 6.40-6.46 (d, 1H, N3-H), 4.80 (s, 1H, N1-H), 8.65 (s, 1H, CH=N), 1.24 (s, 3H, C6-CH_3); Mass (*m/z*): 374(M-1) $^+$, 375(M)$^+$

5-{5-[(4-hydroxybenzylidene)-amino]-1, 3, 4-oxadiazol-2-yl}-6-methyl-4-phenyl-3,4-dihydro pyrimidin- 2 (1H)-one (4d): M.F: $C_{20}H_{17}N_5O_3$; M.Wt.: 375.3; Yield: 92%; M.P: 315–317^{0}C; R_f: 0.78; Elemental analysis (calcd./ found): C(63.99/63.86), H(4.56/4.30), N(18.66/18.75), O(12.79/12.67); IR (KBr cm^{-1}): 3167 (-OH str.), 3438 (NH str.), 3540 (N-H str.), 1346 (C-N str.), 1735 (C=O str.), 1586(C=C str.); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.18-8.20 (m, 10H, Ar-CH), 6.00-8.00 (m, 8H, Ar- CH), 10.00 (1H, OH), 6.45-6.52 (d, 1H, N3-H), 4.78 (s, 1H, N1-H), 8.60 (s, 1H, CH=N), 1.22 (s, 3H, C6-CH_3); Mass (*m/z*): 376(M+1) $^+$

6-methyl-5-{5-[(4-nitrobenzylidene)-amino]-1, 3, 4-oxadiazol-2-yl}-4-phenyl-3,4-dihydro pyrimidin -2(1H)-one (4e): M.F: $C_{20}H_{16}N_6O_4$; M.Wt.: 404.3; Yield: 89%; M.P: 240–3242^{0}C; R_f: 0.67; Elemental analysis (calcd./found):C(59.40/58.99), H(3.99/4.01), N(20.78/ 21.01), O(15.83/14.99); IR (KBr cm^{-1}): 3450 (N-H str.), 1550 (Ar.C-H str.), 1690 (Ar.C-C str.), 1450 (C-N str.), 1470(N-O str.), 1500 (C=C str.), 1250 (C-N str.); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.25-8.34 (m, 10H, Ar-CH), 6.12-8.34 (m, 8H, Ar- CH), 6.34-6.12 (d, 1H, N3-H), 4.32 (s, 1H, N1-H), 8.53 (s, 1H, CH=N), 1.27 (s, 3H, C6-CH_3);Mass (*m/z*): 403(M)$^+$

5-{5-[(4-fluorobenzylidene)-amino]-1, 3, 4-oxadiazol-2-yl}-6-methyl-4-phenyl-3,4-dihydro pyrimidin-2(1H)-one (4f): M.F: $C_{20}H_{16}FN_5O_2$; M.Wt.: 377.3; Yield: 83%; M.P: 221–223^{0}C; R_f: 0.71; Elemental analysis (calcd./found): C(63.65/63.00), H(4.27/4.12), N(18.56/18.32), O(8.48/8.53); IR (KBr cm^{-1}): 3580 (N-H str.), 3051 (C-H str.Ar), 1556 (C=C), 1450 (C-N str.), 1630 (C=N str), 1045 (Ar-F); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.12-8.64 (m, 10H, Ar-CH), 6.23-7.78 (m, 8H, Ar- CH), 6.23-6.90 (d, 1H, N3-H), 4.30 (s, 1H, N1-H), 1.34 (s, 3H, C6-CH_3); Mass (*m/z*): 376(M-1) $^+$, 377(M)$^+$

***In vitro* antimicrobial activity[5]:** By using the agar dilution method, the antimicrobial activities of the synthesized compounds were determined. All bacterias were grown on Mueller-Hinton agar (Hi-media) plates (37^{0}C, 24 hours). The synthesized compounds were subjected to antimicrobial screening by determining zone of inhibition (in mm). The antimicrobial activities of the synthesized compounds were tested against various gram (+ve) and gram (–ve) bacteria and fungus by comparing with standard drug ampicillin and ketoconazole using solvent control. Selected microorganisms for antimicrobial activity were: *Staphylococcus aureus* (NTCC-6571)**,** *Bacillus subtilis (*B2), *Echerichia coli* (TG1)4**,** *Aspergillus niger, Candida albicans.* The standard strains used for the antimicrobial activity were procured from the Assam Down Town University, Guwahati, India. Results of the activities are described in the table 1.

Table 1
Antimicrobial screening of the synthesize scaffold

Compd.	Conc. (µg/ml)	*Zone of inhibition in (mm)				
		Bacteria			Fungi	
		S. aureus	*B. subtilis*	*E. coli*	*A. niger*	*C. albicans*
4a	200	09.94 ± 0.46	12.67 ± 0.25	11.73 ± 0.26	08.64 ± 0.21	08.37 ± 0.26
	100	06.13 ± 1.16	07.51 ± 0.41	06.86 ± 0.29	05.78 ± 0.29	06.76 ± 0.29
4b	200	22.77 ± 0.45	24.35 ± 0.43	18.31 ± 0.41	23.58 ± 0.36	25.92 ± 0.31
	100	15.23 ± 0.20	14.84 ± 0.28	14.38 ± 0.33	14.68 ± 0.38	15.58 ± 0.42
4c	200	09.94 ± 0.46	12.67 ± 0.25	11.73 ± 0.26	12.50 ± 0.39	11.66 ± 0.31
	100	08.43 ± 0.42	09.27 ± 0.43	08.75 ± 0.65	10.87 ± 0.28	08.52 ± 0.31
4d	200	15.08 ± 0.51	14.8 ± 0.29	13.86 ± 0.51	14.16 ± 0.33	15.39 ± 0.40
	100	10.49 ± 0.33	13.05 ± 0.33	11.66 ± 0.28	11.38 ± 0.34	12.46 ± 0.37
4e	200	18.40 ± 0.24	20.69 ± 0.19	17.77 ± 0.38	20.23 ± 0.20	23.68 ± 0.38
	100	12.76 ± 0.27	14.29 ± 0.52	13.41 ± 0.36	13.01 ± 0.38	14.27 ± 0.42
4f	200	16.13 ± 0.38	16.57 ± 0.21	17.46 ± 0.40	17.67 ± 0.21	16.44 ± 0.36
	100	13.44 ± 0.18	14.40 ± 0.30	12.60 ± 0.28	11.38 ± 0.34	12.46 ± 0.37
Cip.	200	26.52 ± 0.38	28.31 ± 0.18	25.83 ± 0.24	NA	NA
	100	19.63 ± 0.21	20.53 ± 0.42	19.02 ± 0.28	NA	NA
Flu.	200	NA	NA	NA	25.75 ± 0.26	27.48 ± 0.62
	100	NA	NA	NA	18.85 ± 0.39	20.84 ± 0.37
DMSO	--------	--------	--------	--------	--------	--------

Cip= Ciprofloxacin; Flu= Fluconazole; n=3

***In vitro* Antioxident Activity[4]**

DPPH radical scavenging activity: The nitrogen centered stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at λ517 nm using SPECORD® 50 plus (analytic jena) spectrophotometer which is purple in color. This property marks it suitable for spectrophotometer studies. 1, 1-diphenyl-2-picrylhydrazine was obtained upon reaction of a radical scavenging antioxidant with DPPH stable free radical. With respect to the number of electrons captured, the result of decolorization is stoichiometric. To measure antioxidant properties, the alteration in the absorbance obtained in this reaction has been used.

The abilities of the compounds to donate electron or the hydrogen atom were measured from the bleaching of the purple colored methanol solution of DPPH radical. The stable radical DPPH was used in the spectrophotometric assay as a reagent. To 4 ml of methanol solution of DPPH 0.004% (w/v), 1 ml of different concentrations of the test compounds (4 and 8, 10 μg/ml) in methanol were added. After a period of 30 minutes incubation at room temperature, the absorbance of the solution was taken against blank at λ 517 nm. As a standard, ascorbic acid was used. The percent of inhibition (*I* %) of free radical formed from DPPH was then calculated by using the following equation:

$$I\ \% = [(A\text{control} - A\text{sample}) / A\text{control}] \times 100$$

where *A*control is the absorbance of the control reaction (which contains methanolic DPPH and ascorbic acid) and *A*sample is the absorbance of the test compound (which contains methanolic DPPH and test compound). Tests were carried out in triplicate. The results were assigned in figure 1a.

Nitric oxide (NO) scavenging activity: The reaction mixture of 6 ml which contains 4 ml of sodium nitroprusside (10 mM), 1 ml of phosphate buffer saline (pH 7.4) and 1 ml of test samples or standard ascorbic acid solution in dimethyl sulphoxide at different concentrations (4, 8, 10 µg/ ml), was incubated at 25^0C for 150 min. After incubation of the mixture, removal of 0.5 mL of reaction mixture was done containing nitrite ion. 1 ml volume of sulphanillic acid reagent was then added to the mixture, mixed well and then to complete the diazotization, the mixture was allowed to stand for 5 mins.1 ml volume of naphthyl ethylene diamine dihydrochloride was then added, mixed and after that for 30 mins, the mixture was allowed to stand in diffused light. A pink colored chromophore was formed and absorbance was read at λ 640 nm using SPECORD® 50 plus (analytic jena) spectrophotometer. Ascorbic acid was used as a standard. Calculation of NO scavenging activity was done by using the following equation:

$$\%\ \text{of scavenging} = [(A\text{control} - A\text{sample}) / A\text{control}] \times 100$$

where *A*control is the absorbance of the control reaction (which contains all reagents and ascorbic acid) and *A*sample is the absorbance of the test compound (which contains all reagents and test compound). Tests were carried out in triplicate. The results are assigned in figure 1b.

Antidiabetic Assays:

DPP-4 Inhibitory Assay[6]: DPP-4 inhibitory activity of test samples was investigated using a DPP-4 inhibitor screening kit. Initially, 30µL of diluted assay buffer and 10µL of diluted human-recombinant DPP-4 enzyme solution were pipetted and mixed into each well of a 96-well plate containing 10µL of samples with different concentrations in dimethylsulfoxide. Next, 50 µL of the diluted fluorogenic substrate Gly-Pro-Aminomethylcoumarine (AMC), was added to initiate the reaction. The 96-well plate was incubated at 37^0C for 30 min. After incubation, the excitation and emission fluorescence of free AMC were measured at 350-360 nm and 450-465 nm respectively by using a microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

Table 2
DPP-4 and α-Amylase inhibitory activities of various pyrimidin-2-one derivatives

Compound	IC_{50} values (µg/ml)	
	DPP-4	**α-Amylase**
4a	165.43±0.78**	158.26±0.68*
4b	245.89±0.76**	235.65±0.56**
4c	210.22±0.09*	201.81±0.34*
4d	223.13±0.07**	222.35±0.23**
4e	190.10±0.07*	189.32±0.67*
4f	176.12±0.56**	165.78±0.97*
Metformin	251.20±0.02*	--------
Acarbose	--------	242.43±0.03*

[*, ** Significant difference from negative control, Metformin and Acarbose (standard), respectively at P<0.005, using Tukey's test aspost ANOVA test. All values are expressed as mean ±standard deviation of triplicates]

For the negative control and positive control wells, dimethylsulfoxide solvent and metformin were used respectively. The percentage of inhibition was then calculated by using the equation (1):

$$\% \text{ Inhibition} = \frac{\text{OD initial activity - OD inhibitor}}{\text{OD initial activity}} \times 100 \quad (1)$$

The results are assigned in table 2.

α-Amylase Inhibitory Assay[22]: The α-amylase inhibition assay was conducted according to the method described by Abdullah et al[2] with slight modifications. Initially, quantities of 40 µL of the test samples with varying concentrations (0.078-5 mg/mL) were mixed with 30 µL of 0.1 M sodium phosphate buffer in a 96-well microplate prior to the addition of 10 µL of α-amylase (1 U/mL) into the wells. The plate was subjected to incubation at 37^0C for 15 min followed by addition of 30 µL of soluble starch (1.0%) and re-incubated at 37 ^{0}C for 30 mins. The reaction in the mixture was halted by adding 30 µL of hydrochloric acid (1.0 M) and 30 µL of iodine reagent.

The absorbance of the mixture was then measured at a wavelength of 620 nm. Acarbose and phosphate buffer were used as the positive and negative controls respectively. The inhibition activity of α-amylase was calculated by using the following equation (2) as follows:

$$\% \text{ Inhibition} = \frac{\text{OD test - OD control}}{\text{OD test}} \times 100 \quad (2)$$

The results are assigned in table 2.

Results and Discussion

Chemistry: Six 5-[5-(substitutedarylidene)-1, 3, 4-oxadiazol-2-yl]-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1H)-one [4 (a-f)] containing certain groups as substituents on the phenyl ringalong with benzylidene were synthesized. Various substituted benzaldehyde were initially treated with 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-6-methyl-4-phenyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidin-2-(1H)-ones in existence of a catalytic volume of glacial acetic acid to give desired compounds. Based on their IR and 1H NMR data, formation of the intermediate was confirmed. Based on their FT-IR, ^{1}H NMR and Mass data, the structures of the synthesized compound were determined.

Antimicrobial activity: *In vitro* antibacterial activity data of 3,4-dihydropyrimidin-2(1H)-one derivatives against tested organisms displayed significant activity with a wide degree of variation. It is found that compounds 4b, 4e and 4f have shown significant antibacterial activity against gram positive bacteria (Table 1). Rest compounds have exhibited significant to substantial activity against the same strain. Compounds showed most significant antibacterial activity against test organism *B. subtilis* and most significant antifungal activity against test organisms *Aspergillus niger* and *Candida albicans*. Among the evaluated compounds, 4b was found to be the most active against all bacteria and fungi as it could inhibit the microbial growth at concentration of 100 µg/ml with zone of inhibition ranging from 14.38-15.58 mm.

When a comparison is made between the compounds 4a and compound 4b, it appears that compounds with higher electronegative groups are more active than the compounds without having any substituent on the first phenyl ring. This was further confirmed by comparing the data for compounds 4a and 4e. When the comparison was made between 4d and 4c, it was observed that the compounds with *para* substitution are more active against microbes than *ortho* substitution. These compounds were found to be active in order as 4b>4e>4f>4d>4c>4a where 4a represented unsubstitution, 4b represented presence of *para* chloro, 4e represented *para* nitro and 4f indicated the fluoro group at *para* position.

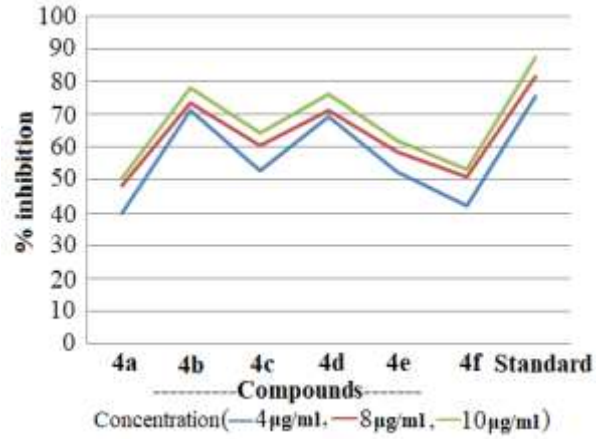
So, from the above result, it has been observed that compounds possessing chloro groups at para position of phenyl ring as in 4b are more active than unsubstituted 4a.

Antioxidant activity (*in vitro*): The compounds 4a to 4f were tested for antioxidant property by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide methods at three different concentrations 4 µg/ml, 8µg/ml and 10µg/ml. The observed data on the antioxidant activity of the compounds-controlled drug are shown in figure 1a and 1b.

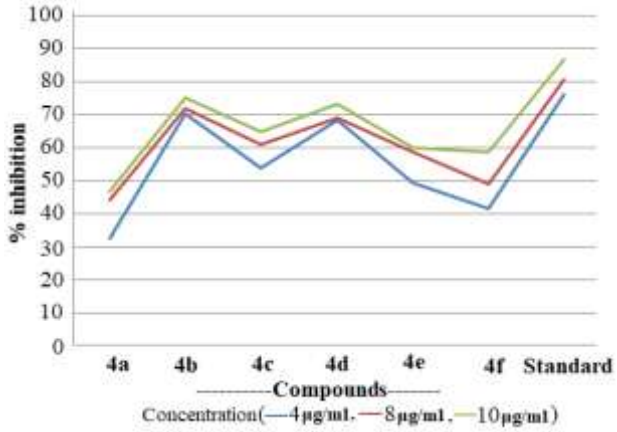
2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method: All the compounds synthesized were tested for antioxidant activity against DPPH free radical. When comparison is made between 4b and 4d at 10µg/ml, presence of the *p*-chloro and *p*-hydroxyl group made the compounds more potent and showed almost equal percentage of inhibition compared to the standard ascorbic acid. Compounds 4c and 4e exhibited comparable percentage of inhibition with the standard. Compound 4f was found to be moderate antioxidant and the compound like 4a was found to be weak antioxidant against DPPH free radical. An increase in concentration results in an increase in DPPH• scavenging activity (Figure 1a).

Nitric oxide scavenging method: Among the compounds tested for antioxidant activity, 4b exhibited the highest antioxidant activity with the % inhibition value of 75.206 while % inhibition of reference compound ascorbic acid was found to be 86.94 (10µg/ml).

Other moderately active compounds 4c and 4d showed the % inhibition values of 64.879and 73.521, respectively. The compounds showed activity which is comparable with control against bacterial strains in increasing order of *p*-Cl >*p*- OH>*o*-OH (Figure 1b).



Values are mean ± SEM (n=3); Standard = Ascorbic acid;
Figure 1a: DPPH Scavenging Method (%DPPH Radical Scavenging activity)



Values are mean ± SEM (n=3); Standard = Ascorbic acid;
Figure 1b: NO Scavenging Method (%NO Radical Scavenging activity)

Antidiabetic activity

Dipeptidyl Peptidase-4 Inhibitory Activity: The DPP-4 inhibitory potential of pyrimidine derivatives is presented in table 4. Significantly, compound 5-{5-[(4-chlorobenzylidene)-amino]-1, 3, 4- oxadiazol – 2 –yl } -6 – methyl – 4 - phenyl-3, 4 dihydropyrimidin -2 (1H) -one (4b) ($p < 0.05$) possessed the excellent inhibition activity against DPP-4 in which the IC50 is 245.89±0.76 µg/ml when compared to the standard compound metformin (IC50: 251.20±0.02) (Table 2) and also showed higher inhibition than the compound 4a (IC50: 165.43±0.78µg/ml) because the electronegative group (-Cl) is present at 1st and 4th position of the phenyl ring.

Preliminary structure-activity relationship revealed that the compounds 5-{5-[(4-hydroxybenzylidene)-amino]-1,3,4-oxadiazol-2-yl}-6-methyl-4-phenyl-3,4-dihydro pyrimidin-2 (1H)-one (4d) with *para*- hydroxy group were found to have excellent activity and compound 4c **(**IC50: 210.22±0.09µg/ml**)** was found to have moderate anti diabetic activity may be due to presence of *ortho*-hydroxy group.

α-Amylase Inhibitory Activity: *α*-amylase is a digestive enzyme responsible for the digestion of carbohydrates. Salivary α-amylase breaks down α-(1, 4)-glycosidic bonds of starch and oligosaccharides into disaccharides during the process of food bolus formation and swallowing.[9] Suppression of post-prandial hyperglycemia can be achieved by inhibition of α-amylase which subsequently reduced the digestion of carbohydrates and reduced glucose absorption into the bloodstream. All the derivatives showed inhibition against α-amylase with compound 4b being the most active derivative with IC50 value of 235.65±0.56 µg/ml ($p < 0.005$), which was comparable to the positive control acarbose (IC50: 242.43±0.03) which may be due to presence of *para*chloro group.

Electron donating moiety ($-CH_3$) was found to be of excellent activity. The other compounds 4d to 4a showed lower inhibition activity with IC50 values ranged from 222.35 to158.26 µg/ml. Compound 4d is a potential inhibitor against α-amylase with an IC50 value of 222.35±0.23 µg/ml ($p < 0.05$), which was comparable to the positive control acarbose (IC50: 242.43±0.03µg/ml) (Table 2).

This is followed by 4c (IC50: 201.81±0.34µg/ml), compound 4e (IC50: 189.32±0.67 µg/ml) and 4f (IC50:165.78±0.97µg/ml) showing relatively milder inhibition. So, from the above results, it has been found that

the test compound without substitution on phenyl ring may show relatively low α-amylase and DPP4 inhibitory activity.

Conclusion

A series of new 3, 4-dihydropyrimidin-2-one derivatives were synthesized and the structures of the entire compound were confirmed by their ^{1}H NMR, Mass and IR spectra. As a result of antimicrobial, antioxident and antidiabetic activity screening, it can be concluded that 3, 4-dihydropyrimidin-2-one derivatives 4a-4f designed in this study had selective action on DPP4 and α-amylase. All the compounds which were synthesized showed moderate effectiveness against bacteria and fungi. The screening studies have demonstrated that the newly synthesized compound, 5-{5-[(4-chlorobenzylidene)-amino]-1, 3, 4- oxadiazol – 2 –yl} -6 – methyl – 4 - phenyl-3, 4 dihydropyrimidin -2 (1H) -one (4b) exhibit promising antimicrobial and antioxidant properties.

SAR observation has shown the importance of electronic environment on antioxidant as well as DPP4 and α-amylase activity. The existence of hydroxyl (OH) and halogens (especially chloro) substituent on the aromatic ring has increased the effectiveness of the compounds compared to those with other substituents which may be due to the existence of the versatile pharmacophore. In conclusion, we feel that the results of preliminary *in vitro* activities of this class of compounds may possess potential for design of new molecules with adaptations on the aryl substituents.

Acknowledgement

The authors are thankful to the Chancellor and Vice chancellor for providing facility to publish this study. The present work was supported by the research cell of Faculty of Pharmaceutical Science, Assam Down Town University.

References

1. Abdel-Aziz A.A.M., El-Azab A.S., El-Subbagh H.I., Al-Obaid A.M., Alanazi A.M. and Al-Omar M.A., Design, synthesis, single-crystal and preliminary antitumor activity of novel arenesulfonylimidazolidin-2-ones, *Bioorg. & Med. Chem. Let.*, **22(5)**, 2008–2014, doi: 10.1016/j.bmcl.2012.01.036 **(2012)**

2. Abdullah N. and Kasim K., *In-Vitro* Antidiabetic Activity of Clinacanthus nutans extracts, *Int. J. of Pharmaco. & Phyto. Res.*, **9**, 846-852, doi:10.25258/phyto.v9i6.8189 **(2017)**

3. Akhter M., Husain A., Azad B. and Ajmal M., Aroylpropionic acid based 2,5-disubstituted-1,3,4-oxadiazoles: synthesis and their anti-inflammatory and analgesic activities, *Euro. J. of Med. Chem*., **44(6)**, 2372–2378, doi: 10.1016/j.ejmech.2008.09.005 **(2009)**

4. Alam F. and Dey B.K., Synthesis, characterization and in-vitro anti-oxidant activity of some novel 1, 3,4-thiadiazole derivatives, *Der. Pharma. Chemica*., **7(1)**, 230-240 **(2015)**

5. Alam F., A Study on the Antimicrobial and Antioxidant Activities ofSome New 1, 3, 4-Thiadiazole Derivatives, *Int. J. of Chem T. Res*., **7(5)**, 2520-2531 **(2015)**

6. Ali Z. et al, Design and synthesis of quinazoline-3,4-(4H)-diamine endowed with thiazoline moiety as new class for DPP-4 and DPPH inhibitor, *Bioorg. Chem*., **71**, 181-191, doi: 10.1016/j.bioorg.2017.02.004 **(2017)**

7. Bhardwaj N., Saraf S.K., Sharma P. and Kumar P., Syntheses, evaluation and characterization of some 1, 3, 4-oxadiazoles as antimicrobial agents, *E-Jour. of Chem.*, **6(4)**, 1133-1138, doi: 10.1155/2009/698023 **(2009)**

8. El-Emam A.A., Al-Deeb O.A., Al-Omar M. and Lehmann J., Synthesis, antimicrobial and anti-HIV-1 activity of certain 5-(1-adamantyl)-2-substituted thio-1,3,4-oxadiazoles and 5-(1-adamantyl)-3-substituted aminomethyl-1,3,4-oxadiazoline-2-thiones, *Bioorg. and Med. Chem.*, **12(19)**, 5107–5113, doi: 10.1016/j.bmc.2004.07.033 **(2004)**

9. Holst J.J., The incretin system in healthy humans: The role of GIP and GLP-1, *Met. Clini. & Exp*., **96**, 46-55, doi: 10.1016/j.metabol.2019.04.014 **(2019)**

10. Kagthara P.R., Shah N.S., Doshi R.K. and Parekh H.H., Synthesis of 2,5-disubstituted 1,3,4-oxadiazoles as biologically active heterocycles, *Ind. J. of Chem*., **38(B)**, 572–576 **(1999)**

11. Khan M.S.Y., Khan R.M. and Drabu S., Anticonvulsant and antibacterial activity of some new 1, 3, 4-oxadiazole derivatives, *Ind. J. of Het. Chem*., **11(2)**, 119-122 **(2001)**

12. Kostova I. and Atanasov P.Y., Antioxidant Properties of Pyrimidine and Uracil Derivatives, *Cur. Org. Chem*., **21(20)**, 2096–2108, doi: 10.2174/1385272820666161025152154 **(2017)**

13. Kucukguzel S.G., Oruc E.E., Rollas S., Sahin F. and Ozbek A., Synthesis, characterization and biological activity of novel 4-thiazolidinones, 1,3,4-oxadiazoles and some related compounds, *Euro. J. of Med. Chem*., **37(3)**, 197–206, doi: 10.1016/s0223-5234(01)01326-5 **(2002)**

14. Kumar D., Sundaree S., Johnson E.O. and Shah K., An efficient synthesis and biological study of novel indolyl-1,3,4-oxadiazoles as potent anticancer agents, *Bioorg. & Med. Chem. Let*., **19(15)**, 4492–4494, doi: 10.1016/j.bmcl.2009.03.172 **(2009)**

15. Mallikarjunaswamy C., Mallesha L., Bhadregowda D.G. and Pinto O., Studies on synthesis of pyrimidine derivatives and their antimicrobial activity, *Arab. J. of Chem*., **10(Supple 1)**, S484-S490, doi: 10.1016/j.arabjc.2012.10.008 **(2017)**

16. Osama M., Ahmed O.M., Hussein A.M. and Ahmed R.R., Antidiabetic and Antioxidant Effects of Newly Synthesized Pyrimido[1,6-A] Pyrimidine Derivatives in Neonatal Streptozotocin-Induced Diabetic Rats, *Med. Chem*., **2(1)**, 020-028, doi: 10.4172/2161-0444.1000108 **(2012)**

17. Padhy A.K., Bardhan M. and Panda C.S., Synthesis and anti-microbial activity of some pyrimidine derivatives, *Ind. J. of Chem.*, **42(B)**, 910–915 **(2003)**

18. Prakash O., Kumar M., Kumar R., Sharma C. and Aneja K.R., Hypervalent iodine(III) mediated synthesis of novel unsymmetrical 2,5-disubstituted 1,3,4-oxadiazoles as antibacterial and antifungal agents, *Euro. J. of Med. Chem*., **45(9)**, 4252–4257, doi: 10.1016/j.ejmech.2010.06.023 **(2010)**

19. Samadhiya S. and Halve A., Synthetic utility of Schiff bases as potential herbicidal agents, *Ori. J. of Chem*., **17(1)**, 119-122 **(2001)**

20. Sengupta P., Mal M., Mandal S., Singh J. and Maity T.K., Evaluation of antibacterial and antifungal activity of some 1, 3, 4 oxadiazoles, *Iran. J. of Pharmaco. & Ther.*, **7(2)**, 165–167 **(2008)**

21. Sridhar S.K. and Ramesh A., Synthesis and Pharmacological Activities of Hydrazones, Schiff and Mannich Bases of Isatin Derivatives, *Bio. & Pharma. Bul*., **24(10)**, 1149–1152, doi: 10.1248/bpb.24.1149 **(2001)**

22. Yavari A. et al, A-Glucosidase and A-Amylase Inhibition, Molecular Modeling and Pharmacokinetic Studies of New Quinazolinone-1,2,3-Triazole-Acetamide Derivatives, *Med. Chem. Res.*, **30**, 702-711, doi: 10.1007/s00044-020-02680-8 **(2021)**

23. Yousif M.N.M., El-Sayed W.A., Abbas H.A.S., Awad H.M. and Yousif N.M., Anticancer Activity of New Substituted Pyrimidines, Their Thioglycosides and Thiazolopyrimidine Derivatives, *J. of App. Pharma. Sci*., **7(11)**, 021-032, doi: 10.7324/JAPS.2017.71104 **(2017)**

24. Zarghi A. et al, Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1, 3, 4-oxadiazoles, *Bio. & Med. Chem. Let*., **15(7)**, 1863-1865, doi: 10.1016/j.bmcl.2005.02.014 **(2005)**.

(Received 10th January 2022, accepted 12th March 2022)