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Novel pyrimidinone derivative as potential promising antimicrobial agent

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Abstract

Some new derivatives of 4-phenyl-5-[5'-(3''-chloro-2''-oxo-4''-substitutedaryl-1''-azetidinyl)-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one and 4-phenyl-5-[5'-(2''-substitutedaryl- 4' '-oxo -1'',3''- thiazolidin -3''-yl) -1',3',4 '-oxadiazol-2'-yl]- 6- methyl-3,4-dihydro pyrimidin -2-one have been synthesized by reacting different substituted Schiff bases with chloroacetyl chloride and thioglycolic acid, respectively. The structures of synthesized compounds were confirmed by IR and ^{1}H-NMR data. Antimicrobial activities of compounds were investigated using the standard method against different bacterial and fungal strains in comparison with standard drugs. Microbiological results indicated that the synthesized compounds possess a board spectrum of activity.

Keywords Pyrimidine, oxadiazolylazetidinone, oxadiazolylthiazolidinone, schiff base, antimicrobial activity.

INTRODUCTION

Antimicrobial agents have a chronicle of success in controlling morbidity due to infectious diseases. But, as a consequence of frequent use, bacterial and fungal resistance to known classes of antimicrobial agent has become a severe global problem in recent years and presents a continuous clinical challenge [1-3]. There are serious concerns that untreatable pathogens may develop at an alarming rate in the near future. Strategies to address this challenge include the design of improved versions of antimicrobial classes already in clinical use and the use of drug combinations. The application of these strategies can be quite successful, but high risk of rapid resistance development remains. Thus there is increasing need for identification of novel structure leads that may be of use in designing new potent and less toxic antimicrobial agents.

Pyrimidinone nuclei have been a source of great interest to organic, medicinal and material scientist over many years, which is present in a number of biologically active organic compounds which exhibit antibacterial [4], antifungal [5], anticancer [6], antiviral [7] anti-inflammatory activities [8] and many more. Furthermore, schiff base [9,10], azetidinone [11,12], thiazolidinone [13,14] have also been found to possess promising antimicrobial activity. In view of these observations, we thought that It would be interesting to synthesize the substituted derivatives starting form pyrimidinone followed by combination of oxadiazolylazetidinone oxadiazolylthiazolidinone in one frame may lead to compounds with interesting antimicrobial profile.

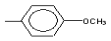
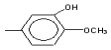
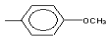
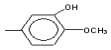
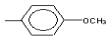
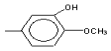
RESULT AND DISCUSSION

Newly synthesized compounds **(4-18) and** standard drug, chlroamphenicol were screened for their antibacterial activity against Escherichia coli **ATCC 25922** (*E. coli*), Bacillus subtilis **ATCC 1633** (*B. subtilis*) and *Staphylococcus aureus* **ATCC 25923** (*S. aureus*) at 250 µg/mL concentration by using diffusion method. All the results of inhibition zone and minimal inhibitory concentration are depicted in Table 1. From the result of antibacterial screening, it was found that all the tested compounds, three compounds exhibited excellent antibacterial activity against different bacterial strains. Among the three compounds, compound **11** showed to be more potent than standard drug against all the bacterial strains with MIC 1.562-6.25 µg/mL (Table 1). Compound **10** and **16** reflected excellent antibacterial activity against *E. coli* and *B. subtilis* with MIC 6.25 and 3.125 µg/mL, respectively. It was also observed from the antibacterial data that compound **9** and **15** displayed the equipotent to the reference drug against the bacterial strains. The rest compounds of this series were less active compared to the standard drug.

The antifungal screening showed that all the tested compounds **(4-18)** showed moderate to good inhibitory growth against Candida albican **ATCC 2091** (*C. albican*), Aspergillus niger **ATCC 9029** (*A. niger*) and Candida krusei **ATCC 6258** (*C. krusei*) at 250 µg/mL concentration. Result of inhibitory zone and minimal inhibitory concentration (Table 1) showed varying degree of antifungal activity of tested compounds. Compound **10, 11** and **16** exhibited higher antifungal activity *C. albican* than standard drug with 1.562-3.125 µg/mL. Further, many compounds (**9, 10, 11, 15** and **16**) displayed equipotent antifungal activity to standard drug, fluconazole.

All the synthesized compounds (**4-18**) were screened for their antimicrobial activity and the result are reported in Table 1. All the compounds showed statically significant antimicrobial activity. Structure activity relationship of these compounds revealed that conversion of different benzylidenes(**4-8**) in to their corresponding azetidinone congers (**9-13**) and thiazolidinone congers (**14-18**) markedly enhanced the antimicrobial activity. It is clear from the results that compound having substituted phenyl group and *p*-aminodimethyl phenyl minimum antimicrobial activity was seen. it is interesting to point out that compounds having *p*-methoxyphenyl group as a substituent, elicited remarkable inhibitory growth. Moreover it has also been observed that *m*-hydroxy-*p*-methoxy phenyl exhibited more potent antimicrobial activity. Compound having *p*-hydroxyl phenyl group, yield less but still adequate antimicrobial activity.

Table 1: Antimicrobial activity of the compounds 4-18 against tested microbial strains

Compound no.	R	Antibacterial activity zone of inhibition (**MIC**)						Antifungal activity zone of inhibition (**MIC**)					
		E. coli ATCC 25922		*B. subtilis* ATCC 1633		*S. aureus* ATCC 25923		*C. albicans* ATCC 2091		*A.niger* ATCC 9029		*C. krusei* ATCC 6258	
4		14	**(>100)**	11	**(>100)**	-	**-**	16	**(50)**	10	**(>100)**	06	**(>100)**
5		17	**(50)**	15	**(25)**	13	**(50)**	21	**(25)**	11	**(>100)**	09	**(50)**
6		23	**(25)**	20	**(12.5)**	16	**(25)**	26	**(12.5)**	15	**(50)**	12	**(25)**
7		09	**(>100)**	-	**-**	-	**-**	11	**(>100)**	-	**-**	-	**-**
8		12	**(>100)**	07	**(>100)**	10	**(>100)**	18	**(50)**	06	**(>100)**	-	**-**
9		25	**(12.5)**	23	**(6.25)**	18	**(25)**	28	**(6.25)**	14	**(50)**	15	**(6.25)**
10		28	**(6.25)**	27	**(3.125)**	20	**(12.5)**	31	**(3.125)**	19	**(25)**	17	**(3.125)**
11		32	**(3.125)**	29	**(1.562)**	25	**(6.25)**	36	**(1.562)**	21	**(12.5)**	20	**(3.125)**
12		19	**(50)**	16	**(25)**	12	**(>100)**	22	**(25)**	11	**(>100)**	-	**-**
13		22	**(25)**	21	**(12.5)**	17	**(25)**	26	**(12.5)**	09	**(>100)**	14	**(12.5)**
14		21	**(25)**	19	**(12.5)**	14	**(50)**	24	**(12.5)**	12	**(50)**	-	**-**
15		27	**(12.5)**	23	**(6.25)**	18	**(25)**	29	**(6.25)**	15	**(50)**	13	**(12.5)**
16		29	**(6.25)**	26	**(3.125)**	21	**(12.5)**	33	**(3.125)**	18	**(25)**	17	**(3.125)**
17		17	**(50)**	14	**(50)**	11	**(>100)**	15	**(50)**	-	**-**	-	**-**
18		20	**(50)**	17	**(25)**	16	**(25)**	21	**(25)**	09	**(>100)**	07	**(>100)**
Chloroamphenicol		26	**(12.5)**	23	**(6.25)**	22	**(12.5)**	-	-	-	-	-	-
fluconazole		-	-	-	-	-	-	29	**(6.25)**	22	**(12.5)**	19	**(3.125)**

. '-' indicate no inhibition.

Therefore, it maybe concluded that

- The differently subsituted benzylidenes (**4-8**) shows mild to moderate antimicrobial activity. cycliztion of these benzylidenes congers (**4-8**) in to their corresponding azetidinone congers (**9-13**) and thiazolidinone congers (**14-18**) enhanced the antimicrobal activity,
- Compound with a phenyl group having methoxy group at para position and hydroxyl at meta position show promising antimicrobial activity.
- Compound **9-13** bearing β-lactam rings possessed better antimicrobial activity corresponding to thiazolidinones **14-18**.

MATERIALS AND METHODS

General procedure for synthesis of 4-phenyl-5-carboethoxy-6-methyl-3,4-dihydropyrimidine-2-one, (**1**) [15]

A mixture of urea (0.05 mol), ethlacetoacetate (0.75 mol) and benzaldehyde (0.5 mol) in ethanol (25 mL) with catalytic amount of concentrated hydrochloric acid (5 drops) were heated to reflux for 3 h. the reaction mixture was cooled to room temperature and poured in to crushed ice. The precipitated solid was filtered, washed with cold water and recrystallized from ethanol. m.p. 185 ^{o}C; yield 81 %; IR (KBr) *v* in cm^{-1} : 3314 (NH), 3048 (C-H aromatic), 2965 (C-H aliphatic), 1680, 1715 (C=O), 1584 (C=C of aromatic ring), 1071 (C-O-C). ^{1}H-NMR ($CDCl_3$) δ in ppm: 9.21 (s, 1H, N*H* exchangeable with D_2O), 8.51 (s, 1H, N*H* exchangeable with D_2O), 7.56-7.67 (m, 5H, Ar-*H*), 6.85 (d, 1H, C*H* of pyrimidinone), 4.12 (q, 2H, $COOCH_2CH_3$), 1.29 (t, 3H, $COOCH_2CH_3$), 1.23 (s, 3H, CH_3). MS [M^{+}] at m/z 260. Anal. Cald for $C_{14}H_{16}N_2O_3$: C, 64.61;H, 6.15; N, 10.76; Found: C, 64.78;H, 6.01; N, 10.62.

General procedure for synthesis of 4-phenyl-5-semicarbazido-6-methyl-3,4-dihydropyrimidine-2-one, (**2**)

Compound **1** (0.01 mol) and semicarbazide hydrochloride (0.01 mol) in ethanol (60 mL) were heated under reflux in the presence of anhydrous sodium hydroxide (4.0 g) for 10 h. the ethanol was distilled off and visous mass poured onto crushed ice, filtered and washed several times with water and finally recrystallized from methanol to afford compound **2**. m.p. 165 ^{o}C; yield 72 %; IR (KBr) *v* in cm^{-1} : 3325 (NH), 3265 NH_2), 3058 (C-H aromatic), 2945 (C-H aliphatic), 1665, 1715 (C=O), 1584 (C=C of aromatic ring), 1085 (N-N), 1052 (C-O-C). ^{1}H-NMR (DMSO-d_6) δ in ppm: 9.23 (s, 1H, N*H* exchangeable with D_2O), 8.53 (s, 1H, N*H* exchangeable with D_2O), 8.35 (m, 4H, N*H*N*H*CONH_2), 7.57-7.66 (m, 5H, Ar-*H*), 6.83 (d, 1H, C*H* of pyrimidinone), 1.23 (s, 3H, CH_3). MS [M^{+}] at m/z 289. Anal. Cald for $C_{13}H_{15}N_5O_3$: C, 53.97; H, 5.19; N, 24.22; Found: C, 53.77; H, 5.01; N, 24.09.

General procedure for synthesis of 4-phenyl-5-(5'-amino-1',3',4'-oxadiazol-2'-yl)-6-methyl-3,4-dihydropyrimidine-2-one, (**3**)

A mixture of compound **2** (0.05 mol) and concentrated sulphuric acid (15 mL) was allow to stand 24 h at room temperature and neutralized with ammonium hydroxide. The product was filtered and washed with cold water. The solid product was recrystallized from methanol-water to give compound **3**. m.p. 152 ^{o}C; yield 65 %; IR (KBr) *v* in cm^{-1} : 3345 (NH), 3254 NH_2), 3048 (C-H aromatic), 2975 (C-H aliphatic), 1685, 1710 (C=O), 1584 (C=C of aromatic ring), 1075 (N-N), 1062 (C-O-C). ^{1}H-NMR ($CDCl_3$) δ in ppm: 9.22 (s, 1H, N*H* exchangeable with D_2O), 8.51 (s, 1H, N*H* exchangeable with D_2O), 7.58-7.67 (m, 5H, Ar-*H*), 6.81 (d, 1H, C*H* of pyrimidinone), 6.21 (brs, 2H, NH_2 exchangeable with D_2O), 1.24 (s, 3H, CH_3). MS [M^{+}] at m/z 259. Anal. Cald for $C_{12}H_{13}N_5O_2$: C, 55.59; H, 5.01; N, 27.02; Found: C, 55.71; H, 5.21; N, 27.21.

General procedure for synthesis of 4-phenyl-5-[5'-(substitutedbenzylidinylimino)-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**4-8**)

To a solution of compound **3** (0.02 mol) in methanol (55 mL), aromatic aldehyde (0.02 mole) along with few drops of glacial acetic acid was added. This resulting mixture was refluxed for 10-12 h, while TLC monitored progress and completion of the reaction. The volatiles were

evaporated, and the remaining mixture was filtered. The solid separated out was crystallized form appropriate solvents to get compounds **4-8**.

Characterization and spectral data of 4-phenyl-5-[5'-(p-hydroxyarylidinylimino)-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**4**)
m.p. 102 ^{o}C; yield 55 %; IR (KBr) *v* in cm^{-1} : 3543 (OH), 3314 (NH), 3038 (C-H aromatic), 2965 (C-H aliphatic), 1685, 1710 (C=O), 1584 (C=C of aromatic ring), 1075 (N-N), 1062 (C-O-C). ^{1}H-NMR (DMSO-d_6) δ in ppm: 10.11 (s, 1H, O*H* exchangeable with D_2O), 9.20 (s, 1H, N*H* exchangeable with D_2O), 8.54 (s, 1H, N*H* exchangeable with D_2O), 8.12 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.15-7.47 (m, 9H, Ar-*H*), 6.80 (d, 1H, C*H* of pyrimidinone), 1.23 (s, 3H, CH_3). MS [M^{+}] at m/z 375. Anal. Cald for $C_{20}H_{17}N_5O_3$: C, 64.00; H, 4.53; N, 18.66; Found: C, 64.12; H, 4.68; N, 18.79.

Characterization and spectral data of 4-phenyl-5-[5'-(p-methoxyarylidinylimino)-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**5**)
m.p. 121 ^{o}C; yield 62 %; IR (KBr) *v* in cm^{-1} : 3325 (NH), 3044 (C-H aromatic), 2965 (C-H aliphatic), 1665, 1680, 1715 (C=O), 1564 (C=C of aromatic ring), 1061 (N-N), 1041 (C-O-C). ^{1}H-NMR ($CDCl_3$) δ in ppm: 9.21 (s, 1H, N*H* exchangeable with D_2O), 8.55 (s, 1H, N*H* exchangeable with D_2O), 8.14 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.14-7.48 (m, 9H, Ar-*H*), 6.81 (d, 1H, C*H* of pyrimidinone), 3.22 (s, 3H, OCH_3), 1.21 (s, 3H, CH_3). MS [M^{+}] at m/z 389. Anal. Cald for $C_{21}H_{19}N_5O_3$: C, 64.78; H, 4.88; N, 17.99; Found: C, 64.91; H, 4.71; N, 17.86.

Characterization and spectral data of 4-phenyl-5-[5'-(m-hydroxy-pmethoxyarylidinylimino)-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**6**)
m.p. 132 ^{o}C; yield 57 %; IR (KBr) *v* in cm^{-1} : 3560 (OH), 3345 (NH), 3068 (C-H aromatic), 2944 (C-H aliphatic), 1665, 1695 (C=O), 1574 (C=C of aromatic ring), 1064 (N-N), 1041 (C-O-C). ^{1}H-NMR (DMSO-d_6) δ in ppm: 10.12 (s, 1H, O*H* exchangeable with D_2O), 9.21 (s, 1H, N*H* exchangeable with D_2O), 8.53 (s, 1H, N*H* exchangeable with D_2O), 8.11 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.25-7.86 (m, 8H, Ar-*H*), 6.81 (d, 1H, C*H* of pyrimidinone), 3.24 (s, 3H, OCH_3), 1.21 (s, 3H, CH_3). MS [M^{+}] at m/z 405. Anal. Cald for $C_{21}H_{19}N_5O_4$: C, 62.22; H, 4.69; N, 17.28; Found: C, 62.41; H, 4.81; N, 17.41.

Characterization and spectral data of 4-phenyl-5-[5'-(arylidinylimino)-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**7**)
m.p. 153 ^{o}C; yield 54 %; IR (KBr) *v* in cm^{-1} : 3314 (NH), 3062 (C-H aromatic), 2965 (C-H aliphatic), 1665, 1690, 1715 (C=O), 1544 (C=C of aromatic ring), 1072 (N-N), 1052 (C-O-C). ^{1}H-NMR ($CDCl_3$) δ in ppm: 9.22 (s, 1H, N*H* exchangeable with D_2O), 8.56 (s, 1H, N*H* exchangeable with D_2O), 8.13 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.45-7.52 (m, 10H, Ar-*H*), 6.84 (d, 1H, C*H* of pyrimidinone), 1.22 (s, 3H, CH_3). MS [M^{+}] at m/z 359. Anal. Cald for $C_{20}H_{17}N_5O_2$: C, 66.85; H, 4.73; N, 19.49; Found: C, 66.71; H, 4.87; N, 19.59.

Characterization and spectral data of 4-phenyl-5-[5'-(p-aminodimethylarylidinylimino)-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**8**)
m.p. 148 ^{o}C; yield 56 %; IR (KBr) *v* in cm^{-1} : 3335 (NH), 3030 (C-H aromatic), 2942 (C-H aliphatic), 1660, 1695 (C=O), 1564 (C=C of aromatic ring), 1524 (C-N-C), 1054 (N-N), 1024 (C-O-C). ^{1}H-NMR (DMSO-d_6) δ in ppm: 9.21 (s, 1H, N*H* exchangeable with D_2O), 8.54 (s, 1H,

N*H* exchangeable with D_2O), 8.11 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.13-7.47 (m, 9H, Ar-*H*), 6.80 (d, 1H, C*H* of pyrimidinone), 2.15 [s, 6H, Ar-N(CH_3)$_2$], 1.21 (s, 3H, CH_3). MS [M^+] at m/z 402. Anal. Cald for $C_{22}H_{22}N_6O_2$: C, 65.67; H, 5.47; N, 20.89; Found: C, 65.81; H, 5.32; N, 20.72.

General procedure for synthesis of 4-phenyl-5-[5'-(3''-chloro-2''-oxo-4''-substitutedaryl-1''-azetidinyl)-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**9-13**)
To a solution of compound (**4-8**, 0.01 mole) in DMF (70 mL), chloroacetyl chloride (0.02 mole) and triethyl amine (0.02 mole) were added at 0-5 ^oC temperature with constant stirring. This reaction mixture was refluxed on water bath for 6-8 h; then excess of solvent was distilled off. The precipitated product was cooled, poured in ice-water then filtered, further recrystallized form appropriate solvents to procure compounds **9-13**.

Characterization and spectral data of 4-phenyl-5-[5'-{3''-chloro-2''-oxo-4''-(p-hydroxyphenyl)-1''-azetidinyl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**9**)
m.p. 116 ^oC; yield 58 %; IR (KBr) *v* in cm^{-1} : 3540 (OH), 3324 (NH), 3048 (C-H aromatic), 2985 (C-H aliphatic), 1665, 1740 (C=O), 1564 (C=C of aromatic ring), 1154 (C-N), 1075 (N-N), 1052 (C-O-C), 785 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ in ppm: 10.11 (s, 1H, O*H* exchangeable with D_2O), 9.21 (s, 1H, N*H* exchangeable with D_2O), 8.55 (s, 1H, N*H* exchangeable with D_2O), 8.14 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.15-7.52 (m, 9H, Ar-*H*), 6.82 (d, 1H, C*H* of pyrimidinone), 6.52 (d, 1H, C*H*-Cl, *J* = 6.5 Hz), 1.20 (s, 3H, CH_3). MS [M^+] at m/z 475.5 and [M+2] at m/z 477.5. Anal. Cald for $C_{24}H_{18}N_5O_4Cl$: C, 58.47; H, 3.98; N, 15.50; Found: C, 58.34; H, 4.11; N, 15.62.

Characterization and spectral data of 4-phenyl-5-[5'-{3''-chloro-2''-oxo-4''-(p-methoxyphenyl)-1''-azetidinyl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**10**)
m.p. 146 ^oC; yield 62 %; IR (KBr) *v* in cm^{-1} : 3344 (NH), 3068 (C-H aromatic), 2952 (C-H aliphatic), 1675, 1735 (C=O), 1570 (C=C of aromatic ring), 1144 (C-N), 1065 (N-N), 1041 (C-O-C), 765 (C-Cl). ^{1}H-NMR ($CDCl_3$) δ in ppm: 9.22 (s, 1H, N*H* exchangeable with D_2O), 8.57 (s, 1H, N*H* exchangeable with D_2O), 8.15 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.18-7.54 (m, 9H, Ar-*H*), 6.83 (d, 1H, C*H* of pyrimidinone), 6.52 (d, 1H, C*H*-Cl, *J* = 6.5 Hz), 3.19 (s, 3H, OCH_3), 1.20 (s, 3H, CH_3). MS [M^+] at m/z 465.5 and [M+2] at m/z 467.5. Anal. Cald for $C_{23}H_{20}N_5O_4Cl$: C, 59.29; H, 4.29; N, 15.63; Found: C, 59.41; H, 4.41; N, 15.46.

Characterization and spectral data of 4-phenyl-5-[5'-{3''-chloro-2''-oxo-4''-(m-hydroxy-p-methoxyphenyl)-1''-azetidinyl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, **(11)**
m.p. 122 ^oC; yield 55 %; IR (KBr) *v* in cm^{-1} : 3530 (OH), 3345 (NH), 3052 (C-H aromatic), 2944 (C-H aliphatic), 1685, 1740 (C=O), 1584 (C=C of aromatic ring), 1154 (C-N), 1075 (N-N), 1055 (C-O-C), 768 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ in ppm: 10.12 (s, 1H, O*H* exchangeable with D_2O), 9.22 (s, 1H, N*H* exchangeable with D_2O), 8.52 (s, 1H, N*H* exchangeable with D_2O), 8.14 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.25-7.82 (m, 8H, Ar-*H*), 6.82 (d, 1H, C*H* of pyrimidinone), 6.55 (d, 1H, C*H*-Cl, *J* = 6.5 Hz), 3.23 (s, 3H, OCH_3), 1.24 (s, 3H, CH_3). MS [M^+] at m/z 481.5 and [M+2] at m/z 483.5. Anal. Cald for $C_{23}H_{20}N_5O_5Cl$: C, 57.32; H, 4.15; N, 14.53; Found: C, 57.46; H, 4.28; N, 14.71.

Characterization and spectral data of 4-phenyl-5-[5'-{3''-chloro-2''-oxo-4''-(phenyl)-1''-azetidinyl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**12**)
m.p. 142 ^{o}C; yield 59 %; IR (KBr) *v* in cm^{-1} : 3342 (NH), 3068 (C-H aromatic), 2964 (C-H aliphatic), 1675, 1734 (C=O), 1584 (C=C of aromatic ring), 1171 (C-N), 1085 (N-N), 1042 (C-O-C), 755 (C-Cl). ^{1}H-NMR ($CDCl_3$) δ in ppm: 9.21 (s, 1H, N*H* exchangeable with D_2O), 8.58 (s, 1H, N*H* exchangeable with D_2O), 8.11 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.44-7.52 (m, 10H, Ar-*H*), 6.83 (d, 1H, C*H* of pyrimidinone), 6.52 (d, 1H, C*H*-Cl, *J* = 6.5 Hz), 1.22 (s, 3H, C*H*$_3$). MS [M$^+$] at m/z 435.5 and [M+2] at m/z 437.5. Anal. Cald for $C_{22}H_{18}N_5O_3Cl$: C, 60.61; H, 4.13; N, 16.07; Found: C, 60.74; H, 4.01; N, 15.95.

Characterization and spectral data of 4-phenyl-5-[5'-{3''-chloro-2''-oxo-4''-(p-aminodimethylphenyl)-1''-azetidinyl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**13**)
m.p. 124 ^{o}C; yield 56 %; IR (KBr) *v* in cm^{-1} : 3322 (NH), 3052 (C-H aromatic), 2974 (C-H aliphatic), 1682, 1742 (C=O), 1574 (C=C of aromatic ring), 1162 (C-N), 1065 (N-N), 1024 (C-O-C), 775 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ in ppm: 9.23 (s, 1H, N*H* exchangeable with D_2O), 8.56 (s, 1H, N*H* exchangeable with D_2O), 8.13 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.16-7.49 (m, 9H, Ar-*H*), 6.81 (d, 1H, C*H* of pyrimidinone), 6.54 (d, 1H, C*H*-Cl, *J* = 6.5 Hz), 1.23 (s, 3H, C*H*$_3$). MS [M$^+$] at m/z 480.5 and [M+2] at m/z 482.5. Anal. Cald for $C_{24}H_{23}N_6O_3Cl$: C, 60.18; H, 4.80; N, 17.55; Found: C, 60.32; H, 4.65; N, 17.68.

General procedure for synthesis of 4-phenyl-5-[5'-(2''-substitutedaryl-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**14-18**)
A solution of compound (**4-8**, 0.02 mole) and thioglycolic acid in dry dioxane (50 mL) in presence of anhydrous ZnCl2 (2 g) was refluxed for 9-11 h. Reaction was routinely followed by TLC. After completion of the reaction, excess of solvent was removed through distillation and solid thus obtained was poured onto crushed ice, then filtered, dried and recrystallized from appropriate solvent to yield the compounds **14-18**.

Characterization and spectral data of 4-phenyl-5-[5'-{2''-(p-hydroxyphenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**14**)
m.p. 156 ^{o}C; yield 60 %; IR (KBr) *v* in cm^{-1} : 3532 (OH), 3324 (NH), 3078 (C-H aromatic), 2974 (C-H aliphatic), 1675, 1725 (C=O), 1564 (C=C of aromatic ring), 1084 (N-N), 1062 (C-O-C), 685 (C-S-C). ^{1}H-NMR ($CDCl_3$) δ in ppm: 10.11 (s, 1H, O*H* exchangeable with D_2O), 9.20 (s, 1H, N*H* exchangeable with D_2O), 8.54 (s, 1H, N*H* exchangeable with D_2O), 8.12 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.15-7.51 (m, 9H, Ar-*H*), 6.81 (d, 1H, C*H* of pyrimidinone), 3.66 (d, 2H, C*H*$_2$ of thiazolidinone), 1.21 (s, 3H, C*H*$_3$). MS [M$^+$] at m/z 449. Anal. Cald for $C_{22}H_{19}N_5O_4S$: C, 58.79; H, 4.23; N, 15.59; Found: C, 58.62; H, 4.36; N, 15.77.

Characterization and spectral data of 4-phenyl-5-[5'-{2''-(p-methoxyphenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**15**)
m.p. 169 ^{o}C; yield 45 %; IR (KBr) *v* in cm^{-1} : 3352 (NH), 3078 (C-H aromatic), 2965 (C-H aliphatic), 1675, 1725 (C=O), 1584 (C=C of aromatic ring), 1075 (N-N), 1034 (C-O-C), 665 (C-S-C). ^{1}H-NMR (DMSO-d_6) δ in ppm: 9.21 (s, 1H, N*H* exchangeable with D_2O), 8.55 (s, 1H, N*H* exchangeable with D_2O), 8.13 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.17-7.51 (m, 9H, Ar-*H*), 6.82 (d, 1H, C*H* of pyrimidinone), 3.67 (d, 2H, C*H*$_2$ of thiazolidinone), 3.21 (s, 3H, OC*H*$_3$), 1.23 (s,

3H, C*H*$_3$). MS [M$^+$] at m/z 463. Anal. Cald for $C_{23}H_{21}N_5O_4S$: C, 59.61; H, 4.53; N, 15.11; Found: C, 59.78; H, 4.39; N, 15.97.

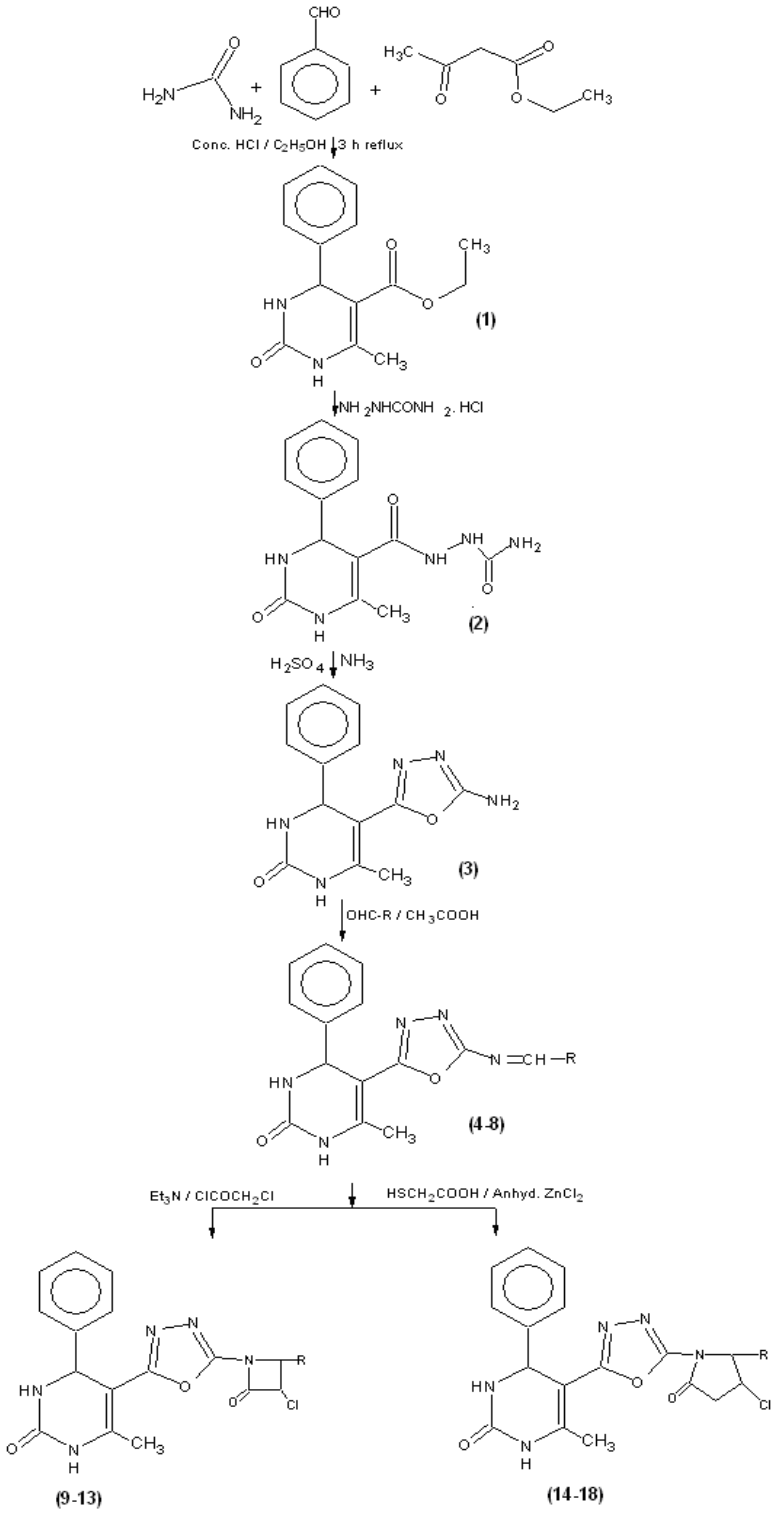
Characterization and spectral data of 4-phenyl-5-[5'-{2''-(m-hydroxy-p-methoxyphenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**16**)
m.p. 121 ^{o}C; yield 42 %; IR (KBr) *v* in cm^{-1} : 3564 (OH), 3340 (NH), 3042 (C-H aromatic), 2974 (C-H aliphatic), 1675, 1720 (C=O), 1574 (C=C of aromatic ring), 1085 (N-N), 1075 (C-O-C), 675 (C-S-C). ^{1}H-NMR (DMSO-d_6) δ in ppm: 10.13 (s, 1H, O*H* exchangeable with D_2O), 9.22 (s, 1H, N*H* exchangeable with D_2O), 8.54 (s, 1H, N*H* exchangeable with D_2O), 8.11 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.25-7.80 (m, 8H, Ar-*H*), 6.79 (d, 1H, C*H* of pyrimidinone), 3.65 (d, 2H, C*H*$_2$ of thiazolidinone), 3.22 (s, 3H, OC*H*$_3$), 1.23 (s, 3H, C*H*$_3$). MS [M$^+$] at m/z 479. Anal. Cald for $C_{23}H_{21}N_5O_5S$: C, 57.62; H, 4.38; N, 14.61; Found: C, 57.78; H, 4.24; N, 14.48.

Characterization and spectral data of 4-phenyl-5-[5'-{2''-(phenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**17**)
m.p. 145 ^{o}C; yield 40 %; IR (KBr) *v* in cm^{-1} : 3342 (NH), 3064 (C-H aromatic), 2965 (C-H aliphatic), 1665, 1724 (C=O), 1580 (C=C of aromatic ring), 1081 (N-N), 1052 (C-O-C), 655 (C-S-C). ^{1}H-NMR ($CDCl_3$) δ in ppm: 9.22 (s, 1H, N*H* exchangeable with D_2O), 8.56 (s, 1H, N*H* exchangeable with D_2O), 8.12 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.42-7.58 (m, 10H, Ar-*H*), 6.81 (d, 1H, C*H* of pyrimidinone), 3.66 (d, 2H, C*H*$_2$ of thiazolidinone), 1.23 (s, 3H, C*H*$_3$). MS [M$^+$] at m/z 433. Anal. Cald for $C_{22}H_{19}N_5O_3S$: C, 60.96; H, 4.38; N, 16.16; Found: C, 61.09; H, 4.50; N, 16.33.

Characterization and spectral data of 4-phenyl-5-[5'-{2''-(p-aminodimethylphenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl}-1',3',4'-oxadiazol-2'-yl]-6-methyl-3,4-dihydropyrimidine-2-one, (**18**)
m.p. 112 ^{o}C; yield 45 %; IR (KBr) *v* in cm^{-1} : 3342 (NH), 3062 (C-H aromatic), 2966 (C-H aliphatic), 1682, 1722 (C=O), 1584 (C=C of aromatic ring), 1065 (N-N), 1034 (C-O-C), 666 (C-S-C). ^{1}H-NMR (DMSO-d_6) δ in ppm: 9.22 (s, 1H, N*H* exchangeable with D_2O), 8.56 (s, 1H, N*H* exchangeable with D_2O), 8.14 (s, 1H, C*H*-Ar, *J* = 11.0 Hz), 7.17-7.52 (m, 9H, Ar-*H*), 6.81 (d, 1H, C*H* of pyrimidinone), 3.68 (d, 2H, C*H*$_2$ of thiazolidinone), 1.21 (s, 3H, C*H*$_3$). MS [M$^+$] at m/z 476. Anal. Cald for $C_{24}H_{24}N_6O_3S$: C, 60.50; H, 5.04; N, 17.64; Found: C, 60.31; H, 5.16; N, 17.79.

Pharmacology
All the compounds (**4-18**) prepared, herein, were screened for antibacterial and antifungal activities against different strains of bacterium and fungus.

Microbiology
The antimicrobial activity was assayed *in vitro* by the twofold broth dilution [16] against bacteria Escherichia coli **ATCC 25922** (*E. coli*), Bacillus subtilis **ATCC 1633** (*B. subtilis*) and *Staphylococcus aureus* **ATCC 25923** (*S. aureus*) and fungus Candida albican **ATCC 2091** (*C. albican*), Aspergillus niger **ATCC 9029** (*A. niger*) and Candida krusei **ATCC 6258** (*C. krusei*). The minimal inhibitory concentrations (MIC, µg/ml) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. All compounds, dissolved in dimethylsulfoxide, were added to culture media .Mueller Hinton Broth for bacteria and Sabouraud Liquid Medium for fungi to obtain final concentrations ranging from 100µg/ml to



Scheme I

R= substituted aryls

1.592 µg/ml. The amount of dimethylsulfoxide never exceeded 1% v/v. Inocula consisted of 5.0 x10^4 bacteria/ml and 1.0 x10^3 fungi/ml. The MICs were read after incubation at 37 °C for 24 h (bacteria) and at 30°C for 48 h (fungi). Media and media with 1% v/v dimethylsulfoxide were employed as growth controls. Chloroamphenicol and fluconazole were used as reference antibacterial and antifungal drugs, respectively. To detect the type of antimicrobial activity, subcultures were performed by transferring 100 µl of each mixture remaining clear in 1 ml of fresh medium. The minimal bactericidal concentrations (MBC, µg/ml) and the minimal fungicidal concentrations (MFC, µg/ml) were read after incubation at 37 °C for 24 h and at 30 °C for 48 h, respectively.

Antibacterial activity

The newly synthesized compounds (**4-18**) were screened for antibacterial activity against bacterial strains namely Escherichia coli **ATCC 25922** (*E. coli*), Bacillus subtilis **ATCC 1633** (*B. subtilis*) and *Staphylococcus aureus* **ATCC 25923** (*S. aureus*) concentration of 250µg/ml by filter paper disc method [17]. For the comparison, chloroamphenicol was used as a standard drug. DMSO served as control and due this there was no visible change in bacterial growth. The discs of Whatmann filter paper were prepared with standard size (7 mm) and kept into 1 Oz screw capped wide mouthed containers for sterilization. These bottles are kept in to hot air oven at 150 ^oC. Now, solution is then put into each bottle. The discs are transferred to the inoculated plates with a pair of fine pointed tweezers. To prevent contamination tweezers may be kept with their tips in 70% alcohol and flamed off before use. Before use the test organism, which were grown on nutrient agar. They were sub cultured in nutrient broth at 37 ^oC for 18-20 h. Carefully each disc was applied to the surface of agar without lateral movement once the surface had been touched. Now the plates incubated for 24 h at 37 ^oC.

Antifungal activity

Newly synthesized compounds (**4-18**) and standard drug, fluconazole were evaluated for antifungal by employing standard agar disc diffusion method [18]. The following strains of fungus have been used in this study: Candida albican **ATCC 2091** (*C. albican*), Aspergillus niger **ATCC 9029** (*A. niger*) and Candida krusei **ATCC 6258** (*C. krusei*). All cultures were maintained on SDA and incubated at 30^oC. To prepare homogeneous suspensions of above fungi for disc assays, they were grown in sabouraud broth centrifuged to collect the pellet and buffered saline. The fungal pellet was homogenized in a sterile hand held homogenizer. This suspension was then plated onto SDA using fungal spreader to obtain an even growth field. Sterile 6 mm Whatmann filter paper impregnated with 250µg/ml concentration of various test compounds and standard drug, fluconazole. These discs were then placed in the centre of each quadrant of an SDA plate. Each plate had one control disc impregnated with DMSO. The plates were incubated at 30ºC. After 48 h, the plates were removed.

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