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Synthesis and Antimicrobial Activity of Novel Indol Compounds Containing 2-azitidinones and 1,3,4 Oxadiazoles

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ABSTRACT

New novel derivatives of 4-(3-(1-((4-acetyl-5-methyl-5-(p-substituted phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro-1-(4 substituted phenyl)azetidin-2-one (5a-g) were prepared by the condensation of acetohydrazide (4a-g) with acetic anhydride. The compound 4(a-g) was obtained by the reaction of (3) with 4-substituted acetophenone in the presence of glacial aceticacid. The synthon (3) was obtained by the reaction of compound(2) with hydrazine hydrate in ethanol. The compound (2) was obtained by the reaction of (1H-indol-1-yl)acetate(1) with monochloroacetyl chloride in the presence of triethylamine in dioxane. The structure of the newly synthesized compounds were charecterized by IR, NMR, Mass and elemental analysis.

Key words: 1,3,4-oxadiazole, acetic anhydride, acetophenone, Monochloro acetyl chloride, antimicrobial activity.

INTRODUCTION

1,3,4,-Oxadiazoles are five membered heterocycles having two nitrogen atoms and one oxygen atom. 1,3,4-oxadiazoles belong to the group of heterocycles that have been attracting attention for last two decades due to their wide range of biological interactions. Some 1,3,4-oxadiazoles substituted witharyl groups at positions 2 and 5 are of significant interest of polymer and material sciences because of their electro chemical properties(Phosphorescence).

1,3,4-oxadiazole derivatives have played a major role in the pharmaceutical chemistry.Literature reveals that a large number of heterocyclic compounds containing the 1, 3, 4-Oxadiazoles ring are associated with diverse pharmacological properties such as anti-inflammatory, antimicrobial, fungicidal and antiviral activity[1-4]. Substituted 1,3,4-oxadiazole have revealed antibacterial[5,6], antitubercular[7], vasodialatory[8], antifungal[9,10], cytotoxic[11], anti-inflammatory and analgesic[12-15], hypolipidemic[16], anticancer[17,18] and ulcerogenic[19]

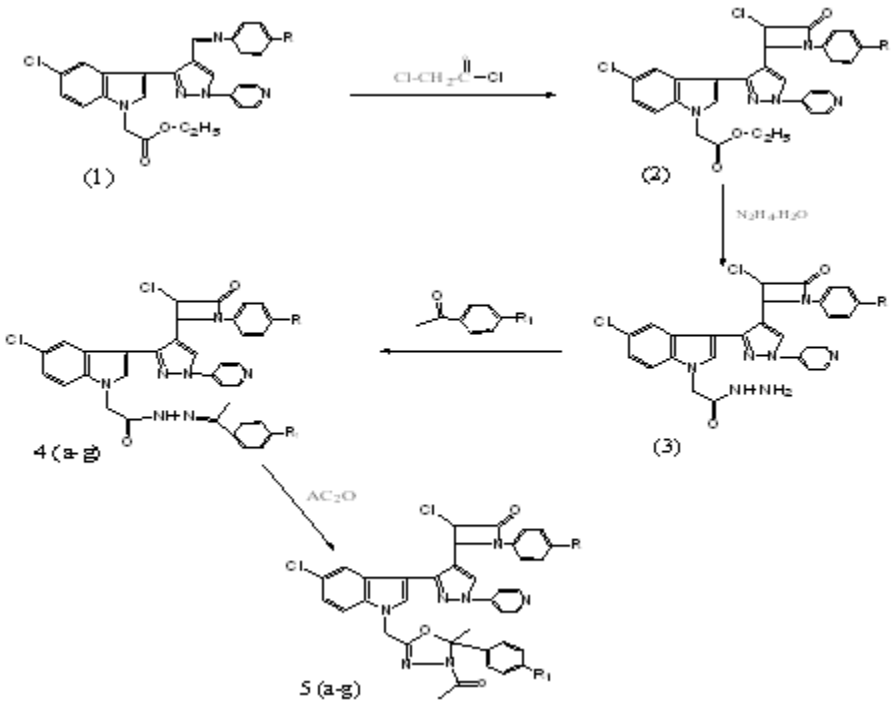
antimycobacterail[20], anticonvulsant[21] activities. We have designed and synthesized new 1,3,4-Oxadiazoles as a potential antimicrobial agent. The results of this study are discussed in this paper.

MATERIALS AND METHODS

All the chemicals used in the present investigation were purchased from Ark pharma, Inc. and Sigma-Aldrich Chemicals company, Inc.USA. and used without further purification. TLC was performed on aluminium sheet of silica gel $60F_{254}$,E-Merk,Germany using iodine as visualizing agent. Melting points were determined in open capillary tubes on Mel-Temp apparatus and are uncorrected (in degree celsius). Columnchromatography was performed on silica gel with different solvent systems as eluents to afford the pure compound. The Infra Red Spectra of the compounds were recorded in KBr pellets on FT-IR(perkin-Elmer 1000 units)instrument . All ^{1}H and^{13}C-NMR spectra were recorded on a varian XL-300 Spectrometer operating at 400MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR. The ^{1}H-NMR spectra were recorded using TMS as an internal standard(Chemical shifts in δ_{ppm}). The compounds were dissolved in DMSO-d_6 and Chemical shifts were referenced to TMS (^{1}H and ^{13}C-NMR). Mass spectral data was recorded on FAB-MS instrument at 70ev with direct inlet system. Elemental analysis were recorded on a Carlo Erba 1108 elemental Analyser, Central Drug Research Institute, Lucknow, India.

Preparation of Intermediates

2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-substituted phenyl)azetidin-2-yl)-1- (pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N'-(1-(p-substitutedphenyl)ethylidene)acetohydrazide (4a-g)



Synthetic route for target compounds

Compound	5a	5b	5c	5d	5e	5f	5g
R1	-H	$-CH_3$	$-OCH_3$	4-Cl	4-Br	$4-NO_2$	$-CF_3$
R	$-CF_3$	$-CF_3$	$-CF_3$	$-CF_3$	$-CF_3$	$-CF_3$	$-CF_3$

A mixture of 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4- substituted phenyl)azetidin-2-yl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetohydrazide(3) (1.62mmol,1g) in hot methanol (10ml), acetophenone (10 mmol) and a drop of glacial acetic acid were added. Resulting reaction mixture was refluxed for 3hrs at room temperature.After completion of the reaction as indicated by TLC. The solid that separated was filtered wash with cold methanol and purified by column chromatography by using hexane: ethylacetate(7:3) used as eluent to afford 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-(trifluoromethyl) phenyl) azetidin-2-yl)-1(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N'-(1-phenylethylidene) acetohydrazide(4a). The reaction procedure leding to (4a) , was then extended to 4(b-g) from (3) reaction with 4-methyl,4-methoxy,4-chloro,4-bromo,4-nitro,4-trifluoromethyl acetophenone to afforded the compounds 4(b-g).

Synthesis of Compounds

Ethyl 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-substitutedphenyl)azetidin-2-yl)-1- (pyridin-4-yl)1H- pyrazol-3-yl)-1H-indol-1-yl)acetate (2)

A mixture of Schiff's Base ethyl2-(5-chloro-3-(1-(pyridin-4-yl)-4-(((4-(trifluoromethyl) phenyl) imino)methyl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (1) (20mmol ,11.03g) in acetone, triethylamine (0.005mol, 0.75 ml) was added. To this, a solution of chloroacetyl chloride (30 mmol, 3.5 ml) was added drop wise with stirring. The Mixture was refluxed up to 8h. The triethyl amine hydrochloride formed was filtered and washed several times with acetone. The filtrate and washings were mixed and concentrated under reduced pressure. The residue obtained was washed with petroleum ether (40-60^{0}C) to remove the unreacted Schiff's base and the solid obtained was recrystallized from ethanol to afford compound(2).

2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-substituted phenyl)azetidin-2-yl)-1-(pyridin-4-yl)1H -pyrazol-3-yl)-1H-indol-1-yl)acetohydrazide (3)

A mixture of Ethyl 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-substitutedphenyl)azetidin-2-yl)-1- (pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (2) (20mmol) and hydrazine hydrate (30mmol) in ethanol 20ml was refluxed for 6-7hours. The reaction mixture was cooled and poured on to

The structures of the newly synthesized compounds were supported by physical data (Table-1) and following spectral analysis

Comp	R	RI	M.P.	Yield (%)	Molecular Formula	Elemental Analysis Found, Calculated(%)				Rf
						C(%)	H(%)	N(%)	O(%)	
5a	-CF3	-H	158-59	65%	$C_{38}H_{28}Cl_2F_3N_7O_3$	60.02 (60.17)	3.67 (3.72)	12.78 (12.93)	6.17 (6.33)	0.60
5b	-CF3	-CH3	152-53	63%	$C_{39}H_{30}Cl_2F_3N_7O_3$	(60.63) 60.47	(3.91) 3.76	(12.69) 12.54	(6.21) 6.04	0.62
5c	-CF3	OCH3	142-44	60%	$C_{39}H_{30}Cl_2F_3N_7O_4$	(59.40) 59.24	(3.83) 3.67	(12.43) 12.28	(8.12) 7.98	0.72
5d	-CF3	4-Cl	165-67	62%	$C_{38}H_{27}Cl_3F_3N_7O_3$	(57.55) 57.40	(3.43) 3.28	(12.36) 12.21	(6.05) 5.90	0.68
5e	-CF3	4-Br	162-63	63%	$C_{38}H_{27}BrCl_2F_3N_7O_3$	(54.50) 54.35	(3.25) 3.10	(11.71) 11.56	(5.73) 5.57	0.63
5f	-CF3	4-NO2	184-86	70%	$C_{38}H_{27}Cl_2F_3N_8O_5$	(56.80) 56.65	(3.39) 3.23	(13.94) 13.77	(9.96) 9.80	0.48
5g	-CF3	4-CF3	178-79	68%	$C_{39}H_{27}Cl_2F_6N_7O_3$	(56.67) 56.52	(3.29) 3.13	(11.86) 11.70	(5.81) 5.65	0.54

ice cold water with stirring. The progress of the reaction was monitored by TLC with hexane:ethyl acetate(7:3) as elutent. The separated solid was filtered, washed with water and recrystallized from ethanol to afford compound (3).

4-(3-(1-((4-acetyl-5-methyl-5-(p-substituted phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl) methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro-1-(4-substitu ted phenyl)azetidin-2-one (5a-g)

A mixture of 4a (1mmol,716.54mg) and excessive acetic anhydride (10ml) was refluxed for 3hrs.The progress of the reaction was monitored by TLC. The excessive acetic anhydride was distilled off and the residue was poured on to crushed ice. The solid thus obtained was filtered and purified by column chromatography by using hexane: ethylacetate (7:3) as eluent to afford compound(5a) (493.07 mg, 0.65mmol). The above cyclisation reaction was then extended to synthesize 5(b-g) from 4(b-g) reaction with acetic anhydride.

RESULTS AND DISCUSSION

The target compounds were synthesized via the route as shown in the Scheme.The synthon required for the synthesis of the target molecules was prepared by a reported method, filtered and recrystallized from ethanol. For all the synthesized compounds, the progress of the reaction was monitored by TLC with cyclohexane, ethylacetate (7:3) as mobile phase. All the synthesized structures showed satisfactory result. The chemical shift values of the synthesized compounds were full agreement with the number of protons present in it.

Physical, Analytical and Spectral data for the synthesized title compounds are given as follows.

Characterization of 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-(trifluoromethyl)phenyl) azetiding-2-yl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N'-(1phenylethylidene) acetohydrazide (4a) yield 65%, M.P:162-63^{0}C, IR (KBR) : (δppm)

Table 2:

Comp	R	R^1	M.P.	Yield (%)	Molecular Formula	Elemental Analysis Found, Calculated(%)				Rf
						C(%)	H(%)	N (%)	O(%)	
4a	4-CF_3	-H	162-63	65%	$C_{36}H_{26}Cl_2F_3N_7O_2$	60.15 (60.34)	3.41 (3.66)	13.57 (13.68)	4.25 (4.47)	0.52
4b	4-CF_3	-CH_3	157-58	62%	$C_{37}H_{28}Cl_2F_3N_7O_2$	60.62 (60.83)	3.54 (3.86)	13.21 (13.42)	4.17 (4.38)	0.57
4c	4-CF_3	OCH_3	148-49	60%	$C_{37}H_{28}Cl_2F_3N_7O_3$	59.34 (59.53)	3.57 (3.78)	12.95 (13.13)	6.26 (6.43)	0.65
4d	4-CF_3	4-Cl	168-69	63%	$C_{36}H_{25}Cl_3F_3N_7O_2$	57.42 (57.58)	3.18 (3.36)	12.88 (13.06)	4.07 (4.26)	0.53
4e	4-CF_3	4-Br	165-67	64%	$C_{36}H_{25}BrCl_2F_3N_7O_2$	54.16 (54.36)	3.02 (3.17)	12.15 (12.33)	3.83 (4.02)	0.55
4f	4-CF_3	4-NO_2	186-87	70%	$C_{36}H_{25}Cl_2F_3N_8O_4$	56.63 (56.78)	3.16 (3.31)	14.58 (14.71)	8.24 (8.40)	0.46
4g	4-CF3	4-CF3	175-76	68%	$C_{37}H_{25}Cl_2F_6N_7O_2$	56.48 (56.64)	3.07 (3.21)	12.35 (12.50)	3.92 (4.08)	0.49

3190 cm^{-1}(–NH), 3041 cm^{-1}(=CH), 1696 cm^{-1}(C=O), 1625cm^{-1}(C=N), 677cm^{-1}(C-Cl) respectively. ^{1}H-NMR (400MHz ,DMSO-d_6) ´ppm**:** 10.90(s,1H,-CONH),8.10(s,1H,Pyrazole),7.75-8.40(m,4H of -C_5H_4N) 7.30-7.70(m,4H,-CH of indol),6.80-7.20(m,9H, of -C_6H_5 and $C_6H_4CF_3$), 5.43(d,1H,-CH of azitidin attached to -Cl), 5.10(d, 1H,CH of azitidin ring),3.65 (s,2H, N-CH_2-CO), 2.25(s,3H, N-CH_3). Mass(m/z) : 715.15 , Anal. Calcd. For $C_{28}H_{20}Cl_2F_3N_7O_2$: C, 60.34%; H, 3.66%; N, 13.68%; O, 4.47% . Found: C 60.15%, H 3.41%, N 13.57%, O 4.25% .

Characterization of 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-(trifluoromethyl)phenyl) azitidin -2-yl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)N'-(1-(p-tolyl)ethylidene) acetohydra zide (4b)

Yield 60 %, M.P: 157-58 ^{0}C, IR (KBR) : (´ppm) 3185 cm^{-1}(–NH), 3042cm^{-1}(=CH), 1685 cm^{-1}(C=O), 1625 cm^{-1}(C=N), 676cm^{-1}(C-Cl) respectively. ^{1}H-NMR (400MHz ,DMSO-d_6) ´ppm**:** 10.89 (s,1H,-CONH),8.09(s,1H,Pyrazole),7.75-8.40(m,4H of -C_5H_4N), 7.30-7.70(m,4H,-CH of indol),6.85-7.15(m,8H, of -C_6H_4 and $C_6H_4CF_3$) ,5.42(d,1H,-CH of azitidin attached to -Cl), 5.11 (d,1H,CH of azitidin ring),3.60(s,2H,N-CH_2-CO), 2.30(s,3H,N-CH_3),1.52(s, 1H,-CH_3).

Mass(m/z) : 729.16 , Anal.Calcd.For $C_{37}H_{28}Cl_2F_3N_7O_2$: C, 60.83%; H, 3.86%; N, 13.42%; O, 4.38%. Found: C 60.62%, H 3.54%, N 13.21%, O 4.17% .

Characterization of 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-(trifluoromethyl)phenyl) azetidin-2-yl)-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)N'-(1-(4-methoxyphenyl) ethylidene) acetohydrazide (4c)

yield 63%, M.P: 148-49 ^{0}C, IR (KBR) : (´Àppm) 3184 cm^{-1}(–NH), 3040cm^{-1}(=CH), 1680cm^{-1}(C=O), 1620 cm^{-1}(C=N), 675 cm^{-1}(C-Cl) respectively. ^{1}H-NMR(400MHz ,DMSO-d_6) δppm**:**10.85 (s,1H,-CONH), 8.07(s,1H, Pyrazole), 7.75-8.40(m,4H of -C_5H_4N),7.30-7.70 (m, 4H,-CH of indol),6.80-7.16(m,8H, of -C_6H_4 and $C_6H_4CF_3$) 5.40(d,1H,-CH of azitidin attached to –Cl), 5.10 (d,1H,CHof azitidin ring),3.55(s,2H, N-CH_2-CO),2.32(s,3H, N-CH_3),3.85 (s,1H,-OCH_3).Mass(m/z) : 745.16 , Anal.Calcd.For $C_{37}H_{28}Cl_2F_3N_7O_3$: C, 59.53%; H, 3.78%; N, 13.13%; O, 6.43%. Found: C 59.34%, H 3.57%, N 12.95%, O 6.26% .

Characterization of 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-(trifluoromethyl)phenyl) azetidin -2-yl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N'-(1-(4-chlorophenyl) ethylidene) acetohydrazide (4d)

yield 67%, M.P: 168-69^{0}C, IR (KBR) : (δ ppm) 3200 cm^{-1}(–NH), 3045cm^{-1}(=CH), 1690cm^{-1}(C=O), 1623 cm^{-1}(C=N), 676 cm^{-1}(C-Cl) respectively. ^{1}H-NMR(400MHz ,DMSO-d_6) δ ppm: 10.92 (s,1H,-CONH),8.08(s,1H,Pyrazole),7.75-8.40(m,4H of -C_5H_4N),7.30-7.70(m,4H,-CH of indol),6.85-7.18 (m,8H, of -C_6H_4Cl and $C_6H_4CF_3$) 5.44(d,1H,-CH of azitidin attached to –Cl) ,5.10 (d,1H,CH of azitidin ring), 3.57 (s,2H, N-CH_2-CO),2.31(s,3H, N-CH_3). Mass(m/z) : 749.11 , Anal. Calcd.For $C_{36}H_{25}Cl_3F_3N_7O_2$: C, 57.58%; H, 3.36%; N, 13.06%; O, 4.26% , Found: C 59.42%, H 3.18%, N 12.88%, O 4.01% .

Characterization of N'-(1-(4-bromophenyl) ethylidene)-2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-(trifluoromethyl)phenyl)azetidin-2-yl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl) acetohydrazide (4e)

yield 66%, M.P:165-67^{0}C, IR (KBR) :(´Àppm)3205cm^{-1}(-NH),3044cm^{-1}(=CH), 1687cm^{-1}(C=O) ,1623 cm^{-1}(C=N), 677 cm^{-1}(C-Cl) respectively. ^{1}H-NMR(400MHz ,DMSO-d_6) δppm: 10.91(s,1H,-CONH),8.09(s,1H,Pyrazole),7.75-8.40(m,4H of -C_5H_4N),7.30-7.70(m,4H,-CH of indol), 6.80-7.20(m,8H, of -C_6H_4Br and $C_6H_4CF_3$) 5.43(d,1H,-CH of azitidin attached to –Cl), 5.10 (d,1H,CH of azitidin ring), 3.58 (s,2H, N-CH_2-CO),2.32(s,3H, N-CH_3). Mass(m/z) : 793.06 , Anal.Calcd.For $C_{36}H_{25}BrCl_2F_3N_7O_2$: C, 54.36%; H, 3.17%; N, 12.33%; O, 4.02 % . Found: C 59.16%, H 3.02%, N 12.15%, O 3.83% .

Characterization of 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-(trifluoromethyl)phenyl) azetidin-2-yl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N'-(1-(4-nitrophenyl) ethylidene)acetohydrazide (4f)

yield 68 %, M.P: 186-87 ^{0}C, IR (KBR): (δppm) 3215cm^{-1}(-NH), 3047cm^{-1}(=CH),1695cm^{-1}(C=O) ,1626cm^{-1}(C=N), 678 cm^{-1}(C-Cl) respectively. ^{1}H-NMR(400MHz ,DMSO-d_6) δppm:10.95 (s, 1H,-CONH),8.10(s,1H,Pyrazole),7.75-8.40(m,4H of -

C_5H_4N),7.30-7.70(m,4H,-CH of indol) ,6.95-7.28(m,8H,of-$C_6H_4NO_2$ and $C_6H_4CF_3$),5.44(d,1H,-CH of azitidin attached to –Cl) ,5.09 (d,1H,CH of azitidin ring),3.69 (s,2H, N-CH_2-CO),2.30(s,3H, N-CH_3). Mass(m/z) : 760.13 , Anal.Calcd.For $C_{36}H_{25}Cl_2F_3N_8O_4$: C, 56.78%; H, 3.31%; N, 14.71%; O, 8.40%. Found: C 56.63%, H 3.16%, N 14.58%, O 8.24% .

Characterization of 2-(5-chloro-3-(4-(3-chloro-4-oxo-1-(4-(trifluoromethyl)phenyl) azetidin-2-yl)-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N'-(1(4(trifluoromethyl) phenyl) ethylidene) acetohydrazide (4g)

yield 70%,M.P:175-76 ^{0}C,IR(KBR): (δppm) 3210 cm^{-1}(–NH), 3046cm^{-1}(=CH), 1694cm^{-1}(C = O) , 1624 cm^{-1}(C = N) , 676 cm^{-1}(C-Cl)respectively.^{1}H-NMR (400MHz, DMSO-d_6) δppm :10.93 (s,1H,-CONH),8.081(s,1H,Pyrazole),7.75-8.40(m,4H of -C_5H_4N),7.30-7.70(m,4H,-CH of indol),6.90-7.25(m,8H, of two -$C_6H_4CF_3$ rings), 5.45(d,1H,-CH of azitidin attached to –Cl) ,5.11 (d,1H,CH of azitidin ring),3.65 (s,2H, N-CH_2-CO),2.35(s,3H, N-CH_3). Mass(m/z) : 783.14 , Anal.Calcd.For $C_{37}H_{25}Cl_2F_6N_7O_2$: C, 56.64%; H, 3.21%; N, 12.50%; O, 4.08%. Found: C 56.48%, H 3.07%, N 12.35%, O 3.92% .

Characterization of 4-(3-(1-((4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro-1-(4(trifluoro methyl) phenyl)azetidin-2-one (5a)

yield 65%, M.P:158-59^{0}C, IR (KBR) : (´ppm) 3042cm^{-1}(=CH(aromatic),),1698cm^{-1} (C=O) ,1620cm^{-1}(C=N), 1140 cm^{-1}(N-N), 678 cm^{-1}(C-Cl) respectively. ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm : 8.10(s,1Hof Pyrazole),7.75-8.43(m,4H of C_5H_4N) 7.30-7.70(m,4H,-CH of indol),6.80-7.25(m,9H, of -C_6H_5 and $C_6H_4CF_3$), 5.45(d,1H,-CH of aziti din attached to -Cl), 5.15(d,1H,-CH of azitidin ring),3.55(s,2H,-NCH_2 attached to indol nucleus), 2.46(s,3H of -$COCH_3$ group), 2.22(s,3H,-CH_3). C^{13}-NMR 400MHz , DMSO-d_6 (´ ppm) : 129, 111,121, 126, 123, 113, 135, 130, 126,129,116, 61, 62, 162, 143, 134, 125,132,124,147, 114, 150, 60, 159, 90, 169, 24, 28, 142, 127, 128.5, 126.5 corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, $C_{12,}$ C_{13}, C_{14}, C_{15}, C_{16}&C_{20}, C_{17} & C_{19}, C_{18}, $C_{21,}$ C_{22}, C_{23} &C_{26},C_{24} &C_{25}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32},C_{33},C_{34}&C_{38},C_{35} &C_{37} and C_{36}carbon atom respectively. Mass(m/z) : 757.16, Anal. Calcd.For $C_{38}H_{28}Cl_2F_3N_7O_3$: C, 60.17%; H, 3.72%; N, 12.93%; O, 6.33%. Found: C 60.02%, H 3.67%, N 12.78%, O 6.17% .

Characterization of 4-(3-(1-((4-acetyl-5-methyl-5-(p-tolyl)4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro-1-(4-(trifluoro methyl) phenyl)azetidin-2-one (5b)

yield 64 %, M.P:152-53^{0}C, IR (KBR) : (δppm) 3042cm^{-1}(=CH(aromatic),),1695cm^{-1}(C=O),1620cm^{-1} (C=N), 1645&1232 cm^{-1}(1,3,4-oxadiazole), 676cm^{-1}(C-Cl) respectively. ^{1}H-NMR (400MHz, DMSO-d_6) δppm : 8.09(s,1H,N-CH gp.),7.75-8.41(m,4Hof C_5H_4N),7.30-7.70(m,4H,-CH of indol),6.85-7.15(m,8H, of -C_6H_4 and $C_6H_4CF_3$) ,5.44(d,1H,-CH of azitidin attached to –Cl), 5.13(d,1H,-CH of azitidin ring),3.52(s,2H,-NCH_2 attached to indolnucleus),2.45(s,3H of -$COCH_3$ group),2.23(s, 3H,CH_3) ,1.57(s,3H,-CH_3 attached to phenyl ring).. C^{13}-NMR 400MHz DMSO-d_6 (´ ppm) : 129.1, 111.2, 121.3, 125.9, 123, 112.7, 134.7, 130, 126, 129, 115.8,61, 62, 162.1 ,142.8, 134, 125.1, 132, 124, 147, 114, 150, 60, 158.2, 90, 168.5, 24, 28, 139,127,128.8,136.5,21. 21.5 corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, $C_{12,}$ C_{13},C_{14}, C_{15}, C_{16}&C_{20}, C_{17}&C_{19}, C_{18}, $C_{21,}$ C_{22},C_{23}&C_{26},C_{24} &C_{25}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33},C_{34}&C_{38},C_{35} &$C_{37,}$ C_{36} and C_{39}carbon atom respectively. Mass(m/z) : 771.17 , Anal. Calcd. For $C_{39}H_{30}Cl_2F_3N_7O_3$: C, 60.63%; H, 3.91%; N, 12.69%; O, 6.21% . Found: C 60.47%, H 3.76%, N 12.54%, O 6.04%

Characterization of 4-(3-(1-((4-acetyl-5-(4-methoxyphenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro-1-(4-(trifluoromethyl)phenyl) azetidin-2-one (5c)

Yield 62 %, M.P:142-44^{0}C, IR (KBR) : (δppm) 3042cm^{-1}(=CH(aromatic),),1680cm^{-1}(C=O),1617cm^{-1} (C=N), 1645&1232 cm^{-1}(1,3,4-oxadiazole), 675cm^{-1}(C-Cl) respectively. ^{1}H-NMR (400MHz, DMSO-d_6) δ ppm: 8.07(s,1H,N-CH gp.),7.75-8.40(m,4Hof C_6H_4N),7.30-7.70(m,4H,-CH of indol),6.80-7.16(m,8H, of -C_6H_4 and $C_6H_4CF_3$), 5.43(d,1H,-CH of azitidin attached to –Cl), 5.12(d,1H,-CH of azitidin ring), 3.50(s,2H,-NCH_2 attached to indol nucleus), 2.46(s,3H of -$COCH_3$group), 2.24(s, 3H,-CH_3),3.82(s,3H,-OCH_3).

C^{13}-NMR 400MHz DMSO-d_6 (´ ppm) : 129.2, 111, 121.9, 125.5, 122.6,113, 134.5,130,125.5, 129, 116, 61, 62, 162.5, 143, 133.9, 125.5, 132, 124.3, 147, 114, 150, 58, 160, 91, 169, 23.9, 28, 135, 128, 114.5, 158.7, 55.8 corresponding toC_1, C_2, C_3, C_4, C_5, C_6, C_7,C_8,C_9,C_{10},C_{11},$C_{12,}C_{13}$,C_{14}, C_{15}, C_{16}&C_{20}, C_{17} & C_{19}, C_{18}, $C_{21,}$ C_{22}, C_{23}&C_{26},C_{24} &C_{25}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, C_{34}& C_{38},C_{35} &$C_{37,}$ C_{36} and C_{39}carbon atom respectively. Mass(m/z) : 787.17 , Anal.Calcd.For $C_{39}H_{30}Cl_2F_3N_7O_4$: C, 59.40%; H, 3.83%; N, 12.43%; O, 8.12%. Found: C 59.24%, H 3.67%, N 12.28%, O 7.98% .

Characterization of 4-(3-(1-((4-acetyl-5-(4-chlorophenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro-1-(4-(trifluoromethyl) phenyl)azetidin-2-one (5d)

Yield 66 %, M.P:165-67^{0}C, IR (KBR) : (δppm) 3042cm^{-1}(=CH(aromatic),),1690cm^{-1}(C=O),1623cm^{-1} (C=N), 1645&1232 cm^{-1}(1,3,4-oxadiazole), 677cm^{-1}(C-Cl) respectively. ^{1}H-NMR (400MHz, DMSO-d_6) δppm: 8.08(s,1H,N-CH gp.),7.75-8.42(m,4Hof C_6H_4N),7.30-7.70(m,4H,-CH of indol),6.85-7.18(m,8H, of -C_6H_4Cl and $C_6H_4CF_3$),5.43(d,1H, -CH of azitidin attached to -Cl), 5.13(d,1H,-CH of azitidin ring), 3.57(s,2H,-NCH_2 attached to indol nucleus), 2.47(s,3H of $COCH_3$ group), 2.24(s,3H,-CH_3). C^{13}-NMR 400MHz DMSO-d_6 (´ ppm) : 129.2, 111.3, 121.7, 125.6, 122.4, 112.4, 134.5, 130.3, 125.4, 129, 115.9, 60.9, 62.1, 162.3, 142.8, 133.9, 125.4, 124.1, 146.9, 113.9, 149.9, 60,158.3, 90.2, 168.5, 23.7, 27.9, 140.7, 125.4,128.7,132.3corresponding to C_1, C_2,C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, $C_{12,}$ C_{13}, C_{14},C_{15}, C_{16}&C_{20}, C_{17}&C_{19}, C_{18}, $C_{21,}$ C_{22},C_{23}&C_{26},C_{24} &C_{25}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, C_{34}&C_{38},C_{35} &$C_{37}$and C_{36}carbon atom respectively. Mass(m/z) : 791.12 , Anal.Calcd.For $C_{38}H_{27}Cl_3F_3N_7O_3$: C, 57.55%; H, 3.43%; N,12.36%; O,6.05% .Found: C 57.40%, H 3.28%, N 12.21%, O 5.90%

Characterization of 4-(3-(1-((4-acetyl-5-(4-bromophenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro-1-(4-(trifluoromethyl) phenyl)azetidin-2-one (5e)

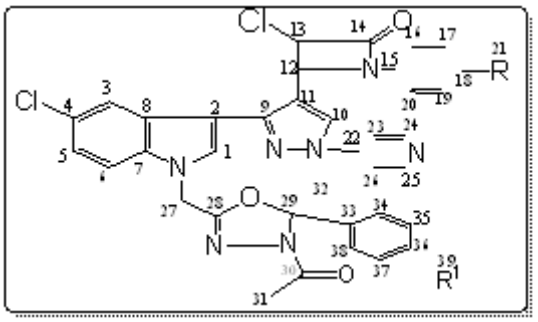
Yield 67 %, M.P:162-63^{0}C, IR (KBR) : (δppm) 3042cm^{-1}(=CH(aromatic),),1688cm^{-1}(C=O),1623cm^{-1} (C=N), 1645&1232 cm^{-1}(1,3,4-oxadiazole), 676cm^{-1}(C-Cl) respectively. ^{1}H-NMR (400MHz, DMSO-d_6) δppm: 8.09(s,1H,N-CH gp.),7.75-8.42(m,4Hof C_6H_4N),7.30-7.70(m,4H,-CH of indol),6.80-7.20(m,8H, of -C_6H_4Br and $C_6H_4CF_3$),5.42(d,1H, -CH of azitidin attached to –Cl), 5.12(d,1H,-CH of azitidin ring), 3.58(s,2H,-NCH_2 attached to indol nucleus), 2.46(s,3H of –$COCH_3$ group), 2.23(s,3H,-CH_3). C^{13}-NMR 400MHz , DMSO-d_6 (δ ppm): 129, 111.2, 121.6, 125.6, 122.4, 112.5, 134.5, 130.2, 125.5, 129,115.9 ,60.9, 62, 162.3, 142.8, 133.8, 125.3, 132.2, 124,147, 114,150, 59,158, 90.2,168.5, 23.9,28, 141.7, 129.2, 131.5, 121.2corresponding toC_1, C_2,C_3,C_4, C_5, C_6, C_7, C_8, C_9, C_{10},C_{11},$C_{12,}C_{13}$,C_{14}, C_{15}, C_{16}&C_{20}, C_{17}&C_{19}, C_{18}, $C_{21,}$ C_{22},C_{23} & C_{26}, C_{24} &C_{25}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, C_{34}& C_{38},C_{35}&C_{37} and C_{36}carbon atom respectively. Mass(m/z) : 835.07 , Anal.Calcd.For $C_{38}H_{27}BrCl_2F_3N_7O_3$: C, 54.50%; H,3.25%;N,11.71%; O, 5.73 % .Found: C 54.35%, H 3.10%, N 11.56%, O 5.57%.

Characterization of 4-(3-(1-((4-acetyl-5-methyl-5-(4-nitrophenyl)-4,5-dihydro-1,3,4 oxadiazol -2-yl)methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro -1-(4-(trifluoro methyl)phenyl)azetidin-2-one (5f)

yield 70 %, M.P:184-86^{0}C, IR (KBR) : (´ppm) 3042cm^{-1}(=CH(aromatic),),1697cm^{-1}(C=O),1625cm^{-1} (C=N), 1645&1232 cm^{-1}(1,3,4-oxadiazole), 678cm^{-1}(C-Cl) respectively. ^{1}H-NMR (400MHz, DMSO-d_6) δppm: 8.10(s,1H,N-CH gp.),7.75-8.45(m,4Hof C_6H_4N),7.30-7.70(m,4H,-CH of indol),6.95-7.28(m, 8H, of-$C_6H_4NO_2$ and $C_6H_4CF_3$),5.44(d, 1H, -CH of azitidin attached to –Cl), 5.14(d,1H,-CH of azitidin ring),3.71(s,2H,-NCH_2 attached to indol nucleus), 2.46(s,3H of –$COCH_3$group) , 2.20(s,3H,-CH_3). C^{13}-NMR 400MHz , DMSO-d_6 (δppm): 129.2, 111.3, 121.7, 125.7, 122.5, 112.5, 134.6, 130.2, 125.4, 129, 115.8, 61, 62.1, 162.2, 142.8, 133.8, 125.3,132.2,124.2,146.9, 113.9, 149.9, 60, 158.2, 90.2, 168.7, 23.8, 27.9, 148.7,127.8,123.7,145.9 corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7,C_8,C_9,C_{10},C_{11},$C_{12,}C_{13}$,C_{14}, C_{15}, C_{16}&C_{20}, C_{17}&C_{19}, C_{18}, $C_{21,}$ C_{22}, C_{23} & C_{26},C_{24} &C_{25}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, C_{34}&C_{38},C_{35} &C_{37} and C_{36}carbon atom respectively. Mass(m/z) : 802.14 , Anal.Calcd.For $C_{38}H_{27}Cl_2F_3N_8O_5$: C, 56.80%; H, 3.39%; N, 13.94%; O, 9.96%. Found: C 56.65%, H 3.23%, N 13.77%, O 9.80%.

Characterization of 4-(3-(1-((4-acetyl-5-methyl-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro-1-(4-(trifluoromethyl) phenyl)azetidin-2-one(5g)

Yield 68 %, M.P:178-79 ^{0}C, IR (KBR) : (δppm) 3042cm^{-1}(=CH(aromatic),),1698cm^{-1}(C=O),1624cm^{-1} (C=N), 1645&1232 cm^{-1}(1,3,4-oxadiazole), 677cm^{-1}(C-Cl) respectively. ^{1}H-NMR (400MHz, DMSO-d_6) δppm: 8.08(s,1H,N-CH gp.),7.75-8.44(m,4Hof C_6H_4N),7.30-7.70(m,4H,-CH of indol),6.90-7.25(m,8H, of two -$C_6H_4CF_3$ rings), 5.45(d,1H, -CH of azitidin attached to –Cl), 5.14(d,1H,-CH of azitidin ring), 3.68(s,2H,-NCH_2 attached to indol nucleus), 2.47(s,3H of –$COCH_3$group), 2.18(s,3H,-CH_3).C^{13}-NMR 400MHz ,DMSO-d_6 (δppm): 129.3, 111.4, 121.7, 125.5, 122.4, 112.5, 134.6, 130.2, 125.4, 129, 115.8, 60.9, 62, 162.2, 142.8,133.8, 125.3, 132.1, 124.1, 146.9, 113.9, 149.9, 60, 158.2, 90.2, 168.5, 23.7, 27.9, 145.9, 127.2, 124.9, 129,124.2 corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15},C_{16} & C_{20},C_{17} & C_{19},C_{18},C_{21}, C_{22}, C_{23} & C_{26}, C_{24} &C_{25}, C_{27},C_{28},C_{29}, C_{30},C_{31},C_{32}, C_{33},C_{34}& C_{38}, C_{35} &C_{37}, C_{36} andC_{39} carbonatom respectively. Mass(m/z):825.15 , Anal. Calcd. For $C_{38}H_{27}Cl_2F_6N_7O_3$: C, 56.67%; H, 3.29%; N, 11.86%; O, 5.81%. Found: C 56.52%, H 3.13%, N 11.70%, O 5.65%.



Biological activity

The newly synthesized compounds 4-(3-(1-((4-acetyl-5-methyl-5-(p-substituted phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-5-chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4 -yl)-3-chloro-1-(4-substituted phenyl)azetidin-2-one (5a-g), were screened for their antimicrobial studies against antibacterial and antifungal activity by Disc Diffusion method[22]. The synthesized compounds were used at the concentration of 250¼g/ml and 500 ¼g/ml using DMF as a solvent [23]. The amoxicillin 10 ¼g/disc and cefaclor 30 ¼g/disc were used as a standard .Whatman No.1 filter paper disk of 5mm diameter were sterile nutrient agar at 45^{o}C.

The sterile disks were impregnated with different compounds synthesized compounds (250¼g/ml). The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 25 ^{o}C for 1 h. To permit good diffusion and then transferred to an incubator at 37 ^{o}C for 48 h.for bacteria , and at 28 ^{o}C for 72 h. For yeast and fungi. The incubation zones aused by the various compounds on the microorganisms were examined. The results of the preliminary screening test are listed in table-3.

Antibacterial activity

The antibacterial activity of 5(a-g) were screened against the *Staphylococus aureus* (gram positive), *Bacillus cerus*, *Escherichia coli* (gram negative) and *Pseudomonas aeruginosa* organisms. In a given series of compounds having nitro (5f) and trifluoromethyl (5g) exhibit high bacterialactivity[24,25] when compared to other substituents. The structural activity relationship for different substituents is in the order i.e. –NO_2 > -CF_3 > -Cl > -Br > -H > -CH_3 > -OCH_3 . Here amoxicillin and cefaclor are tested as reference compounds to compare the activity. The antibacterial activity of 5(a-g) was shown in the below given table.

Antifungal activity

The antifungal activity of final compounds 5(a-g) were screened aginst aspergillus niger ,Candida albicans . In a given series of compounds containing trifluoro methyl and nitro groups in their structures has shown increased effect on their antifungal activity . The structural activity relationship for different substituents is in the order i.e. –NO_2 > -CF_3 > -Cl > -Br > -H > -CH_3 > -OCH_3 Here ketoconazole is tested as reference compound to compare the antifungal activity. Antifungal activity of 4-(3-(1-((4-acetyl-5-methyl-5-(p-substituted phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-5chloro-1H-indol-3-yl)-1-(pyridin-4-yl)-1H-pyrazol-4-yl)-3-chloro-1-(4-substituted phenyl) azetidin -2-one (5a-g) was shown in the below given table.

Table 3:

S. No.	Compd.	Zone of Inhibition (mm)					
		Anti bacterial activity			Anti fungal activity		
		Staphylococus aureus **NCCS 2079**	***Bacillus cereus*** **NCCS 2106**	***Escherichia Coli*** **NCCS 2065**	***Pseudomonas aeruginosa*** **NCCS 2200**	***Aspergillus niger*** **NCCS 1196**	***Candida albicans*** **NCCS 3471**
1)	5a	11	10	11	11	13	15
2)	5b	10	09	10	09	12	13
3)	5c	08	08	09	08	11	11
4)	5d	13	12	11	13	14	19
5)	5e	12	11	10	12	13	16
6)	5f	17	16	15	16	19	21
7)	5g	16	14	13	15	18	20
8)	Amoxicillin	21	27	24	22	-	
9)	Cefaclor	19	22	19	20	-	-
10)	Ketoconazol	-	-	-	-	23	26

CONCLUSION

Indol bearing pyrazole ring, besides azitidinone moiety and the 1,3,4-oxadiazole group were prepared by acetic anhydride reaction with acetohydrazid group. These synthons were purified & charecterized by chromatographic and spectral techniques. Indol derivatives were subjected to antimicrobial evaluation and some of these compounds were found to posses good anti bacterial and anti microbial activity.

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