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Research Article

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Synthesis, characterization, biological evaluation and docking studies of organo phosphorous pyrazole-5-one derivatives of 1,3,4-oxadiazole derivatives of sortase A staphylococcus inhibitors

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ABSTRACT

New novel derivatives of diethyl (1-(4acetyl-5-methyl-5-phenyl-4, 5-dihydro-1,3, 4-oxadiazole-2-yl)methyl)-5-oxo-3-(triflouromethyl)-4,5-dihydro-1h-pyrazol-4-yl)(phenyl/4-methoxy/4-triflouromethyl/4-nitrophenyl amino) methyl phosphate4(a-d) containing various heterocyclic substituent's were synthesized, characterized by elemental analysis,IR,[1]HNMR, [13]CNMR,[31]PNMR spectra and evaluated for in antimicrobial and antifungal activity. Molecular docking studies were performed to calculate docking scores and to propose the binding mode of 1,3,4-oxadiazole.

Keywords: pyrazole 5-one, 1,3,4-oxadiazole, Antimicrobial and Antifungal activity, docking studies of sortase A Staphylococcus inhibitors.

INTRODUCTION

The chemistry of phosphorous heterocyclic compounds containing nitrogen plays an important role in the development of new pharmaceutical materials with novel properties [1,2]. the organo phosphorous compounds and their derivatives were found to be the high light of study in lead compound discovery and biological screening and study of their various biological activities including its application in the field of agricultural, medicinal and industry [3,4]. Organophosporous compounds occupied a unique position in biological activity such as anti bacterial [5], herbicides, insecticides, pesticides [6,7], antifungal agents [8], anti-HIV[9], anti-cancer [10], anti-viral and anti-inflammatory[11].

Heterocyclic compounds represents an important class of biologically active molecules specifically, those containing the pyrazolone nucleus have been shown to posses high biological activities such as tranquillizing, muscle relaxant, psychoanaleptic, anticonvulsant, antihypertensive, antidepressant activities. The derivatives of pyrazolone are important class of antipyretic and analgesic compounds [12-21].

Some substituted pyrazolones and their derivatives are used as antitumor [22], antibacterial [23], antifungal, antiviral, antiparasitic, anti-tubercular and insecticidal agents [23-28], some of these compounds have also anti inflammatory [29], anti-diabetic [30], and anesthetic [31] properties.

A good deal of importance was given to 1,3,4-oxadiazoles and their derivatives in the field of organo phosphorous heterocyclic chemistry due to their unique biological applications [31]. In view of the above observations, we

synthesized pyrazole 5-one possessing 1,3,4-oxadiazole and screening for possible biological, pharmacological activities and docking sortase A staphylococus inhibitory activity by silico methods.

EXPERIMENTAL SECTION

All the chemicals used in the present investigation were purchased from sigma-Aldrich chemicals company, Inc. USA. And used without further purification. TLC was performed on aluminum 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. Column chromatography was performed on silica gel with different solvent systems as eluents to afford the pure compounds. The IR Spectra were recorded as KBr pellets on Perkin –Elmer 1000units, instruments. All ^{1}H-NMR 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. ^{31}P-NMR spectra were recorded on a Varian XL-spectrometer operating at 161.89 MHz The compounds were dissolved in DMSO-$d_6$and chemical shifts were referenced to TMS (H and C-NMR) and 85% H_3PO_4(^{31}P-NMR). Mass spectral data was recorded on a Carlo Erba 1108 elemental analyser, Central drug Research Institute, Lucknow, India.

Docking method

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). This method allows as partial flexibility of protein and full flexibility of ligand. The compounds are docked to the active site of the Sortase A. The interaction of these compounds with the active site residues are thoroughly studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A° (dH-X) for hydrogen bonds and 6.0 A° for vanderwaals were employed. During docking, the default algorithm speed was selected and the ligand binding site in the Sortase A was defined within a 10 A° radius with the centroid as CE atom of PHE136. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5A° RMSD. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each ligand was selected.

RESULTS AND DISCUSSION

Typical procedure of the Synthesis of 2-(5-oxo-4- (phenyl/ 4-methoxy/ 4-nitro/ triflouro methylphenylamino)methyl)-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1-phenylethylidene)acetohydrazide 2(a-d).

A mixture of (R) -2-(5-oxo-4-(phenylimino) methyl)-3-(triflouromethyl)-4, 5-dihydro-1H-Pyrazole-yl)acetohydrazide(1a) and Acetophenone was refluxed in methanol containing a catalytic amount of glacial acetic acid for 4hours.After usual work up the hydrazone 2-(5-oxo-4-(phenylamino)methyl)-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1phenylethylidene) acetohydrazide 2(a) was obtained in 70% yield, m.p 139-142^{0}c.

The structure of these newly synthesized compounds 2(a-d) were based on the characterized by their elemental analysis and spectral data (^{1}H NMR, IR and Mass).

Typical procedure of the Synthesis of (1-(4acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazole-2-yl)methyl)-4-(phenyl/4-methoxy/4-nitro/4-triflouromethylphenylamino)methyl-3-(triflouromethyl)-1H-pyrazol-5(4H)-one3(a-d).

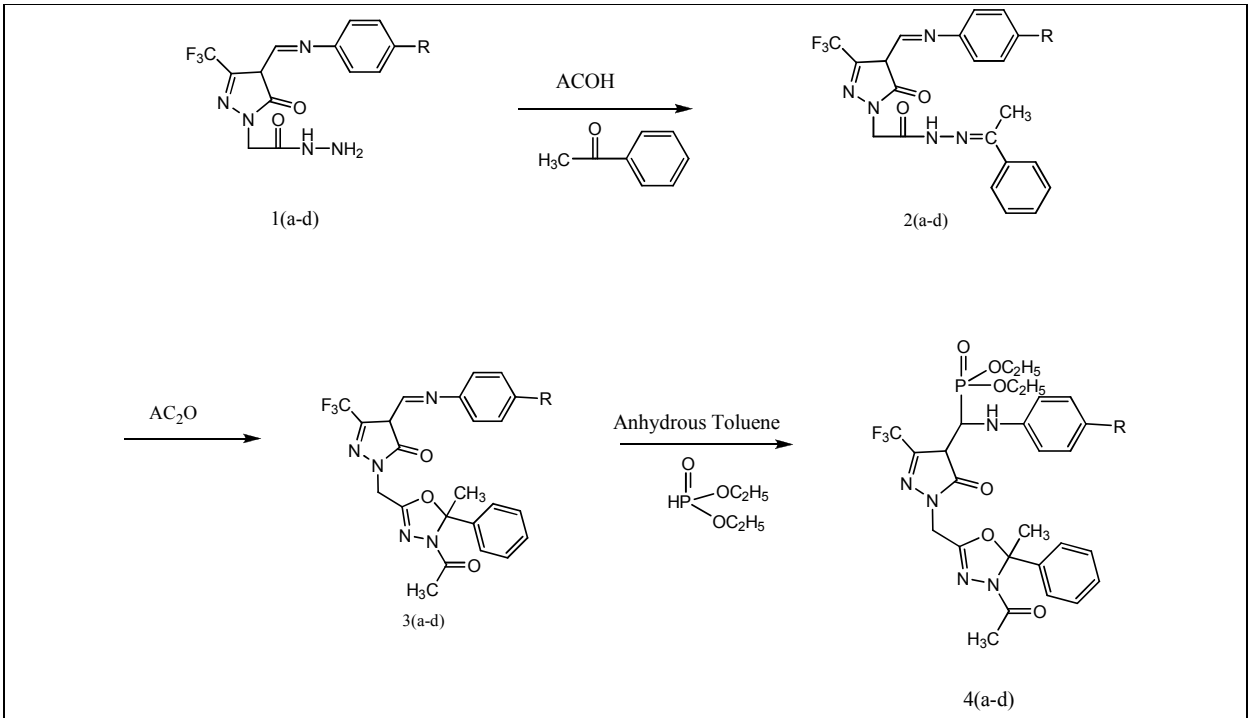
A mixture of hydrazones of 2-(5-oxo-4-(phenylamino) methyl)-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1phenylethylidene)acetohydrazide 2(a) and excess of acetic anhydride was refluxed for 2hours. The acetic anhydride was distilled off, and the reaction mass was poured on to crushed Ice. The solid thus obtained was filtered and recrystallized from aqueous DMF to give (1-(4acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazole-2-yl)methyl)-4-(phenylamino)methyl-3-(triflouromethyl)-1H-pyrazol-5(4H)-one3(a)in yield 70% m.p 151-153^{0}c.

The procedure was adopted to synthesize (3b-d) by the reaction between 2(a-d) with acetic anhydride. The structure of these newly synthesized compounds 3(a-d) were characterized by their elemental analysis and spectral data (^{1}H-NMR and IR).

Typical procedure of the Synthesis of diethyl (1-(4acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazole-2-yl)methyl)-5-oxo-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-4-yl)(phenyl/4-methoxy/4-nitro/4-triflouro methylphenylamino) methylposphate4(a-d):

A mixture of (1-(4acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazole-2-yl)methyl)-4-(phenylamino)methyl-3-(triflouromethyl)-1H-pyrazol-5(4H)-one3(a) and diethyl phosphate(0.50ml,0.004 mol)in an hydrous toluene(15ml) was added drop wise. Stirring was continued at room temperature for another 0.5 hour, after which the mixture was heated under reflux for 4-6 hours. The reaction was monitored by TLC on silica gel using petroleum ether-ethyl acetate(1:2v/v).After completion of the reaction, the solvent was removed by rota evaporator and the resulting residue was purified by column chromatography on silicagel(100-200 mesh)and ethyl acetate-hexane,(3:7 ratio) as an eluent afforded pure,diethyl(1-(4acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazole-2-yl)methyl)-5-oxo-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-4-yl)(phenyl amino)methylphosphate 4(a)was purified from aqueous dimethyl formamide. Yield 70%, mp 173-175^{0}C.

The similar procedure was adapted to synthesis (4a-d) by the reaction between (3b-d) with diethyl phosphate. The structures of these newly synthesized compounds of (4a-d) were established by IR, ^{1}H-NMR, ^{13}C-NMR, ^{13}P-NMR, mass data and elemental analysis.



Scheme.1: synthesis of diethyl(1-(4acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazole-2-yl)methyl)-5-oxo-3-(triflouromethyl)-4,5-dihydro-1h-pyrazol-4-yl)(phenyl/4-methoxy/4-nitro /4-triflouromethyl phenyl amino)methylphosphate 4(a-d)

COMPOUND NUMBER	1a	1b	1c	1d
	2a	2b	2c	2d
	3a	3b	3c	3d
	4a	4b	4c	4d
R	H	OCH_3	NO_2	CF_3

Physical, analytical and spectral data of compounds (2a-d)

Synthesis of 2-(5-oxo-4-(phenylamino) methyl)-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1-phenylethylidene) aceto hydrazide 2(a):

Yield (70%); m.p (139-142); IR (KBr, cm^{-1}) 3210(N-H), 3040(Ar-H), 1698(C=O), 1620(C=N); ^{1}HNMR(400MHz, DMSO-d_6): 2.45(s, 3H, -CH_3group), 3.32(d,1H,-CH of pyrazole ring), 4.20(s,2H,-N-CH_2-C=O) 7.50(d,1H,-CH=N), 6.98-7.52(m,10H,2(C_6H_5)groups), 8.61(s,1H,(O=C-NH);Anal.calcd(%) for $C_{21}H_{20}F_3N_5O_2$: C 58.74%,H 4.23% and N 16.31% . Found: C 58.34%,H 3.83% and N 16.01%.

Synthesis of 2-(5-oxo-4-(4-methoxyphenylamino) methyl)-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1-phenylethylidene) aceto hydrazide 2(b):
Yield(70%);m.p(121-122); IR(KBr,cm^{-1})3218(N-H), 3040(Ar-H), 1692(C=O), 1621(C=N); ^{1}HNMR(400MHz, DMSO-d_6):2.45(s,3H,-CH_3group), 3.32(d,1H,-CH of pyrazole ring),3.83(s,3H,-O-CH_3),4.20(s,2H,-N-CH_2-C=O), 7.50 (d,1H,-CH=N),6.99-7.94(m,9H, $C_6H_{4\&}C_6H_5$), 8.61(s,1H,(O=C-NH);Anal.calcd(%) for $C_{22}H_{22}F_3N_5O_3$: C 57.51%,H 4.39% and N 15.24% . Found: C 57.41%, H 4.09% and N 14.84%.

Synthesis of 2-(5-oxo-4-(4-nitrophenylamino) methyl)-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1-phenylethylidene) aceto hydrazide 2(c):
Yield (70%); m.p (138-140); IR (KBr,cm^{-1}) 3221(N-H),3042(Ar-H),1696(C=O), 1615(C=N); ^{1}HNMR(400MHz, DMSO-d_6):2.45(s,3H,-CH_3group),3.32(d,1H,-CH of pyrazole ring),4.20(s,2H,-N-CH_2-C=O)7.50(d,1H,-CH=N), 6.91-8.10(m, 9H, $C_6H_{4\&}C_6H_5$),8.61(s,1H,(O=C-NH);Anal.calcd(%) for $C_{21}H_{19}F_3N_6O_4$: C 53.17%,H 3.61% and N 17.72% . Found: C 52.87%, H 3.61% and N 17.32%.

Synthesis of 2-(5-oxo-4-(4-triflourophenylamino) methyl)-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1-phenylethylidene) aceto hydrazide 2(d):
Yield (70%); m.p (159-160); IR (KBr,cm^{-1})3213(N-H),3040(Ar-H),1692(C=O), 1617(C=N); ^{1}HNMR(400MHz, DMSO-d_6): 2.45(s,3H,-CH_3group),3.32(d,1H,-CH of pyrazole ring),4.20(s,2H,-N-CH_2-C=O)7.50(d,1H,-CH=N), 7.26-7.94(m,9H,$C_6H_{4\&}C_6H_5$)groups),8.61(s,1H,(O=C-NH);Anal.calcd(%) for $C_{22}H_{22}F_3N_5O_3$: C 57.51%,H 4.39% and N 15.24% . Found: C 57.41%, H 4.09% and N 14.84%.

Physical, analytical and spectral data of compounds (3a-d)
Synthesis of (1-(4acetyl-5-methyl-5-phenyl-4, 5-dihydro-1,3,4-oxadiazole-2-yl)methylphenylamino)methyl-3-(triflouromethyl)-1H-pyrazol-5(4H)-one3(a):
Yield(70%);m.p(151-153);IR(KBr,cm^{-1})3040(Ar-H),1698(C=O),1620(C=N);^{1}HNMR(400MHz,DMSO-d_6): 1.83(s, 3H, -CH_3 group attached to oxadiazole),2.04(s,3H,-CO-CH_3),3.30(d,1H,-CH of pyrazole ring), 4.20(s,2H,-N-CH_2-), 6.98-7.45(m,10H,2(C_6H_5)groups);Anal.calcd(%) for $C_{23}H_{22}F_3N_5O_3$: C 58.60%,H 4.28% and N 14.86% . Found: C 58.20%, H 3.88% and N 14.86%.

Synthesis of (1-(4acetyl-5-methyl-5-phenyl-4, 5-dihydro-1,3,4-oxadiazole-2-yl)methyl (4-methoxyphenyl amino)methyl-3-(triflouromethyl)-1H-pyrazol-5(4H)-one3(b):
Yield(70%);m.p(167-169);IR(KBr,cm^{-1})3040(Ar-H),1692(C=O),1621(C=N); ^{1}HNMR(400MHz, DMSO-d_6): 1.83(s, 3H,-CH_3 group attached to oxadiazole),2.04(s,3H,-CO-CH_3),3.30(d,1H,-CH of pyrazole ring), 4.20(s,2H,-N-CH_2-), 6.85-7.45(m,9H,Ar-H of C_6H_5&C_6H_4groups) ;Anal.calcd(%) for $C_{24}H_{24}F_3N_5O_4$: C 57.48%,H 4.42% and N 13.97% . Found: C 57.08%, H 4.02% and N 13.57%.

Synthesis of (1-(4acetyl-5-methyl-5-phenyl-4, 5-dihydro-1,3,4-oxadiazole-2-yl)methyl (4-nitrophenyl amino) methyl-3-(triflouromethyl)-1H-pyrazol-5(4H)-one3(c):
Yield(65%);m.p(187-189);IR(KBr,cm^{-1})3042(Ar-H),1696(C=O),1615(C=N); ^{1}HNMR(400MHz, DMSO-d_6): 1.83(s, 3H, -CH_3 group attached to oxadiazole),2.04(s,3H,-CO-CH_3),3.30(d,1H,-CH ofpyrazolering),4.20(s,2H,-N-CH_2-),6.72-7.45(m,9H,Ar-HofC$_6H_5$&C_6H_4groups).;Anal.calcd(%) for $C_{23}H_{21}F_3N_6O_5$: C 53.49%,H 3.71% and N 16.27% . Found: C 53.19%, H 3.31% and N 15.19%.

Synthesis of (1-(4acetyl-5-methyl-5-phenyl-4, 5-dihydro-1,3,4-oxadiazole-2-yl)methyl (4-triflourophenyl amino) methyl-3-(triflouromethyl)-1H-pyrazol-5(4H)-one3(d):
Yield(65%); m.p(165-167); IR(KBr,cm^{-1})3040(Ar-H),1692(C=O),1617(C=N); ^{1}HNMR(400MHz, DMSO-d_6): 1.83(s,3H,-CH_3 group attached to oxadiazole),2.04(s,3H,-CO-CH_3),3.30(d,1H,-CH of pyrazole ring), 4.20(s,2H,-N-CH_2-), 6.53-7.45(m,9H,Ar-H of C_6H_5&C_6H_4groups);Anal.calcd(%) for $C_{24}H_{21}F_6N_5O_3$: C 53.44%,H 3.55% and N 12.98% . Found: C 53.14%, H 3.15% and N 12.58%.

Physical, analytical and spectral data of compounds (4a-d)
Synthesis of diethyl (1-(4acetyl-5-methyl-5-phenyl-4, 5-dihydro-1,3, 4-oxadiazole-2-yl) methyl)-5-oxo-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-4-yl)(phenylamino)methylphosphate 4(a):
Yield(70%); m.p(173-175);IR(KBr,cm^{-1})3040(Ar-H),1698(C=O),1620(C=N),1245(P=O), 1053 (O-C),743(P-O); ^{1}HNMR (400 MHz, DMSO-d_6): 1.29 (t, 6H ,-CH_3 groups),1.83(s,3H,-$CH_3$of oxadiazole ring), 2.05(s,3H,-CO-CH_3),2.7(d,1H,-CH of pyrazole ring),2.9(d,1H,-CH attached to pyrazole),4.0(s,1H,Ar-NH),4.10(q,4H,2(O-CH_2-

groups),4.20(s,2H,-N-CH_2-),6.83-7.38(m,10H, Ar-H,2(C_6H_5)groups);^{13}CNMR 75MHz, DMSO-d_6 δppm):115.6, 13.3,176.6,125.5,57.5,62.2,16.3, 52.5,158.2,90.2,27.9,142.6,126.9,128.5,128.7,168.5,23.7,147.6,113.5,129.5,120.8 and these signals are due to C_1 , C_2 , C_3, C_4, C_5 C_6&C_8 ,C_7,&C_9, C_{10}, C_{11}, C_{12}, C_{13} C_{14},C_{15} & C_{19},C_{16}&C_{18}, C_{17},,C_{20},C_{21},C_{22},C_{23} &C_{27},C_{24} &C_{26},and C_{25} Carbon atoms ;^{31}P-NMR (161.89MHz, DMSO-d_6): 17.5; Anal.calcd(%) for $C_{27}H_{31}F_3N_5O_6P$: C 53.20%,H 5.13% and N 11.49% . Found: C 52.80%, H 4.73% and N 11.19%.

Synthesis of diethyl (1-(4acetyl-5-methyl-5-phenyl-4, 5-dihydro-1,3, 4-oxadiazole-2-yl) methyl)-5-oxo-3-(triflouromethyl)-4,5-dihydro-1H-pyrazol-4-yl)(4-methoxyphenylamino) methyl phosphate 4(b):
Yield(70%);m.p(139-141);IR(KBr,cm^{-1})3040(Ar-H),1692(C=O),1621(C=N),1253(P=O),1047 (O-C), 743(P-O); ^{1}HNMR (400 MHz, DMSO-d_6): 1.29(t,6H,-CH_3 groups),1.83(s,3H,-CH_3 of oxadiazole ring),2.05(s,3H,-CO-CH_3),2.7(d,1H,-CH of pyrazole ring),2.9(d,1H,-CH attached to pyrazole),3.83(s,3H,-O-CH_3),4.0(s,1H,Ar-NH), 4.10 (q,4H, 2(O-CH_2- groups),4.20(s,2H,-N-CH_2-),6.83-7.38((m,9H,of C_6H_5&C_6H_4groups); ^{13}CNMR 75MHz, DMSO-d_6δppm): 115.6,13.3,176.6,125.5,57.5,62.2,16.3,52.5,158.2,90.2,27.9,142.6,126.9,128.5,128.7,168.5,23.7, 139.9, 115.8, 115.1, 151.7,55.8 and these signals are due to C_1 , C_2 , C_3, C_4, C_5 C_6&C_8 ,C_7,&C_9, C_{10}, C_{11}, C_{12}, C_{13} C_{14},C_{15}&C_{19},C_{16}&C_{18},C_{17},,C_{20}, C_{21},C_{22},C_{23} &C_{27},C_{24} &C_{26},and C_{25},C_{28} Carbon atoms: Anal.calcd(%) for $C_{28}H_{33}F_3N_5O_7P$: C 52.58%,H 5.20% and N 10.95% . Found: C 52.18%, H 4.90% and N 10.55%.

Synthesis of diethyl (1-(4acetyl-5-methyl-5-phenyl-4, 5-dihydro-1,3, 4-oxadiazole-2-yl) methyl)-5-oxo-3-(triflouromethyl)-4,5-dihydro-1h-pyrazol-4-yl)(4-nitrophenylamino) methyl phosphate 4(c):
Yield(70%);m.p(121-122);I R(KBr,cm^{-1}) 3042(Ar-H), 1696(C=O), 1615(C=N), 1248(P=O), 1038 (O-C),749(P-O); ^{1}HNMR (400 MHz, DMSO-d_6): 1.29(t,6H,-CH_3groups),1.83(s,3H,-$CH_3$of oxadiazole ring),2.05(s,3H,-CO-CH_3),2.7(d,1H,-CH of pyrazole ring),2.9(d,1H,-CH attached to pyrazole),4.0(s,1H,Ar-NH),4.10(q,4H,2(O-CH_2-groups),4.20(s,2H,-N-CH_2-),6.72-8.04(m,9H,Ar-H of C_6H_5&C_6H_4groups);^{13}CNMR 75MHz,DMSO-d_6 δppm):115.6,13.3,176.6,125.5,57.5,62.2,16.3,52.5,158.2,90.2,27.9,142.6,126.9,128.5,128.7,168.5, 23.7,153.7,114.4, 127.5, 136.3 and these signals are due to C_1 , C_2 , C_3, C_4, C_5 C_6&C_8 ,C_7,&C_9, C_{10}, C_{11}, C_{12}, C_{13} C_{14},C_{15}&C_{19}, C_{16}&C_{18}, C_{17},,C_{20}, C_{21},C_{22},C_{23} &C_{27},C_{24} &C_{26},and C_{25} Carbon atoms ^{31}P-NMR (161.89MHz, DMSO-d_6):20.9 Anal.calcd(%) for $C_{27}H_{30}F_3N_6O_8P$: C 49.55%,H 4.62% and N 12.84% . Found: C 49.25%, H 4.22% and N 12.54%.

Synthesis of diethyl (1-(4acetyl-5-methyl-5-phenyl-4, 5-dihydro-1, 3, 4-oxadiazole-2-yl) methyl)-5-oxo-3-(triflouromethyl)-4,5-dihydro-1h-pyrazol-4-yl)(4-triflouromethylphenyl amino) methyl phosphate 4(d):
Yield(68%);m.p(151-153);IR(KBr,cm^{-1})3040(Ar-H),1692(C=O),1617(C=N),1241(P=O),1042 (O-C), 750(P-O); ^{1}HNMR (400 MHz, DMSO-d_6): 1.29(t,6H,-CH_3 groups),1.83(s,3H,-CH_3 of oxadiazole ring),2.05(s,3H,-CO-CH_3),2.7(d,1H,-CH of pyrazole ring),2.9(d,1H,-CH attached to pyrazole),4.0(s,1H,Ar-NH),4.10(q,4H,2(O-CH_2-groups),4.20(s,2H,-N-CH_2-),6.53-7.40((m,9H, Ar-H of C_6H_5&C_6H_4groups);^{13}CNMR 75MHz, DMSO-d_6 δppm):115.6,13.3,176.6,125.5,57.5, 62.2,16.3,52.5,158.2,90.2,27.9,142.6,126.9,128.5,128.7,168.5, 23.7, 150.9, 113.8, 125.9, 124.9,124.1 and these signals are due to C_1 , C_2 , C_3, C_4, C_5 C_6&C_8 ,C_7,&C_9, C_{10}, C_{11}, C_{12}, C_{13} C_{14},C_{15}&C_{19},C_{16}&C_{18},C_{17},,C_{20}, C_{21},C_{22},C_{23} &C_{27},C_{24} &C_{26},and C_{25},C_{28} Carbon atoms ^{31}P-NMR (161.89MHz, DMSO-d_6): 19.6Anal.calcd(%) for $C_{28}H_{30}F_6N_5O_6P$: C 49.64%,H 4.46% and N 10.34% . Found: C 49.44%, H 4.06% and N 10.04%.

Biological activity:
Antimicrobial activity of these newly synthesized compounds was performed according to disc diffusion method, as recommended by the national committee of clinical laboratory. The synthesized compounds were used at the concentration of 250 µg/ml DMF as a solvent.

Antimicrobial activity:
Antibacterial activity
Antibacterial activity Organo phosphorus pyrazole 1,3,4-oxadiazoles **(4a-d)** reported in the exhibit moderate antibacterial activity against the *Staphylococcus aureus* NCCS 2079, *Bacillus Cerus* NCCS 2106, *Escherichia coli* NCCS 2065 and *Pseudomonas aeruginosa* NCCS 2200 at the concentration of 250µg/disc. In this series structures consisting of **4c,** have shown increased effect on their antibacterial activity. The decreasing Oder of antibacterial activity of **(4a-d)** is as follows **"4c>4d>4b>4a".**

Table 1: Antibacterial activity of newly synthesized compounds 4a-d

Comp no	R	Zone of inhibition (mm)			
		Staphylococu Saureus NCCS2079 250(µg/ml)	***Bacillus Cerus NCCS2106 250(µg/ml)***	***Escherichia Coli NCCS2065 250(µg/ml)***	***Pseudomonas aeruginosa NCCS2200 250(µg/ml)***
4a	H	18	13	15	16
4b	OCH_3	19	14	16	17
4c	NO_2	22	18	20	21
4d	CF_3	20	16	18	19
Amoxicilline		27	24	22	25

Antifungal activity

Antifungal activity Organo phosphorus pyrazole 1,3,4-oxadiazoles **(4a-d)**reported in the exhibited moderate antifungal activity against the *Aspergillus niger*NCCS1196 and *Candida albicans* NCCS 3471 at the concentration of 250µg/disc In this series structures consisting of **4c** and have shown increased effect on their antifungal activity. The decreasing Oder of antifungal activity of **(4a-d)** is as follows **"4c>4d>4a>4b".**

Table 2: Antifungal activity of newly synthesized compounds (4a-d)

Com no	R	Zone of inhibition (mm)	
		Aspergillus niger NCCS1196 250(µg/ml)	***Canadidaalbicans NCCS 3471 250(µg/ml)***
4a	-H	13	12
4b	$-OCH_3$	11	9
4c	-NO2	18	16
4d	-CF3	15	13
Ketoconazole		22	25

Docking studies of Organo Phosphorus Pyrazole5-one 1,3,4-oxadiazole (4a-d)

Synthesis and characterization of (1-(4acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazole-2-yl)methyl)-5-oxo-3-(triflouromethyl)-4,5-dihydro-1h-pyrazol-4-yl)(Phenyl/4-methoxy/4-triflouro/4-nitrophenylamino)methyl posphate 4(a-d),

Table.3 Docking results of 4(a-d) on sortase A staphylococcus

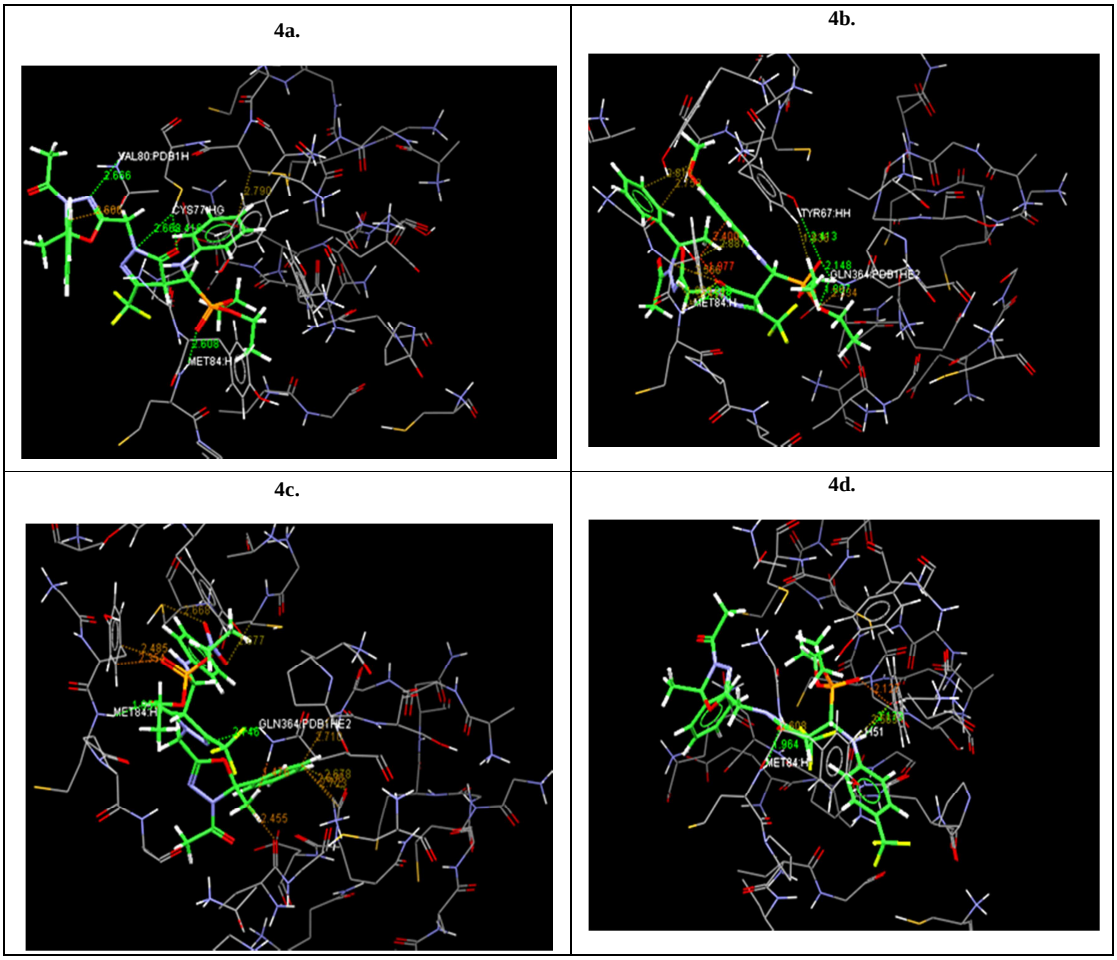
Com no	R	Fittness	S(hb-ext)	S(vdw-ext)	S(hb-int)	S(int)
4a	H	19.02	1.00	29.08	0.00	-21.96
4b	-OCH3	24.84	1.96	30.77	0.00	-19.43
4c	-NO2	37.45	0.55	37.77	0.00	-15.02
4d	-CF3	28.27	0.18	32.20	0.00	-16.18

The docking studies of 4(a-d) were carried out on sortase A staphylococcus (PDB ID: 1T2P). The docking ligands were found to have some interactions between an oxygen atom of the ligands and sortase A staphylococcus protein. Moreover, these docked conformations formed hydrogen bond interactions with the active site of the protein. Bind pocket, common hydrogen bonding interactions were for formed between all the docked ligands and TYR89,CYS77,GLY82PDB, MET84, GLN364PDB, VAL80PDB.The order of protein-ligand hydrogen bond score is 4b>4a>4c>4d.

Besides hydrogen bonding interaction between ligand-protein, the vanderwalls interactions between ligand-protein were also noticed. The order of protein-ligand vanderwaals score of interaction with the protein. However the ligand fails to exhibit intramolecular hydrogen bonding with the ligand. The ligands exhibit minimum intramolecular strain. Finally, all the ligands exhibit moderate to good antimicrobial activity with sortase A staphylococuus protein. The order of gold score fitness value of the ligands is4c>4d>4b>4a. According to gold score fitness value ligand 4c exhibits high binding activity with the protein and ligand 4cshowed leads binding activity with the protein

Table 4: Hydrogen bonding interactions of compounds (4a-d)with sortase A staphylococcus

COMP NO	R	Number of hydrogen bonds	Atom		Bond Length (A^0)	Fitness
			protein	atom		
4a	-H	3	VAL80PDB CYS77 MET84	8(P=O) 5(C=O) 22(C-O)	2.666 2.663 2.608	19.02
4b	$-OCH_3$	3	TYR67 GLN364PDB MET84	8(P=O) 5(C=O) 22(C-O)	2.413 2.148 1.807	24.84
4c	-NO2	2	GLN364PDB MET84	8(P=O) 5(C=O)	2.446 1.954	37.45
4d	-CF3	1	MET84	8(P=O)	1.964	28.27



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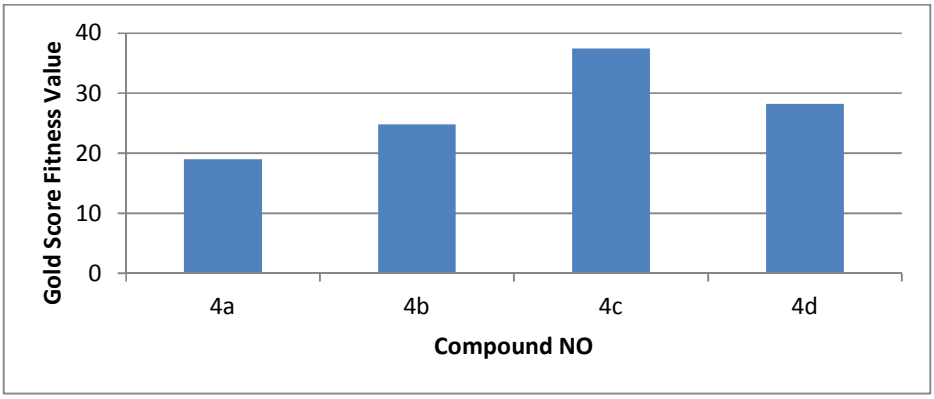

Comparative Gold score fitness values for compounds 4(a-d)

CONCLUSION

The newly synthesized compounds Organo phosphorous pyrazole 5-one containing 1,3,4-Oxadiazole 4(a-d) were found to be active in the study of anti-bacterial and anti-fungal activity. It can be concluded that this class of compounds certainly holds great promise to discover novel classes of antimicrobial agents.

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