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Design, synthesis, characterisation, molecular docking and anti-microbial evaluation of novel 2-(4-substituted) phenoxy-1,3,2-benzodioxaphosphole-2-oxide derivatives containing 1,2,3,4-tetrazol-benzoxozole system as anti-bacterial agents

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ABSTRACT

The present work to develop new anti-bacterial agents, design, synthesis, anti-microbial activities and molecular docking study of 2-(4-substituted) phenoxy-1,3,2-benzodioxaphosphole-2-oxide derivatives containing 1,2,3,4-tetrazol-benzoxozole system. Eight compounds (9a-h) having tetrazol-benzoxozole system were synthesized by chemical methods. The structures of these analogues (9a-h) have been established by ^{1}H NMR, IR, Mass spectral data and elemental analysis. After conformation anti-microbial activity was evaluated by using disc diffusion method. Among eight analogues (9a-h) only three analogues (9d,9e,9f) exhibited significant anti-microbial activity. The synthesized analogues were docked into binding sites of sortase A and evaluated and few analogues shown better activity.

Keywords:

Polyphosphoric acid, substituted phenylphosphorodichloridate, benzoxazole, tetrazole., sortase A, molecular docking.

INTRODUCTION

On inspection of benzoxazoles literature revealed that they possess a wide applications in medicinal chemistry, pharmacology, material chemistry, organometallic and coordination chemistry. Benzoxazoles are found in a variety of natural products[1] and are important targets in drug discovery[2-4]. The targets containing benzoxazole moiety, either isolated from plants or accessed by total synthesis, have remarkable biological activities[5] such as antimicrobial[6], antihistaminic[7], antiparasitics[8], herbicidal[9], antiviral[10], antiallergic[11]. They also found a number of optical applications such as photoluminescents[12], whitening agents[13], as dye lasers[14] and several therapeutic materials[15,16].

Tetrazole is used as gas generating agent for air bags. Several tetrazoles are used as pharmaceutical agents. Tetrazoles can act as pharmacophore for the carboxylate group. Tetrazoles and its derivatives are associated with a variety biological activities such as antifungal[17], antinociceptive[18-19], anticonvulsant[20],antidiabetic[21], cyclo-oxygenaseinhibitors[22], hypoglycaemic[23], antibacterial[24] and anti–inflammatory[25]activities. Tetrazoles are used as catalysts in the synthesis of phosphonates.

Prompted by the above observations, a research project was undertaken to synthesize a series of organophosphorous heterocycles baring benzoxozole and tetrazol moieties in the same carbon skeleton structure.

Thus different 1-(benzo[d]oxazol/thiazole/imidazole/-2-yl)methyl)-4-(1-(2-oxido-2-(4-substituted) phenoxybenzo[d][1,3,2]dioxaphosphol-5-yl)-1h-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (9a-h) were synthesized. The structures of these analogues have been established by Mass, NMR, IR studies, elemental analysis and synthesis.

MATERIALS AND METHODS

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 aluminium sheet of silica gel 60F254, E-Merk, Germany using iodine as visualizing agent. Melting points were determined in open capillary tubes on Mel-Temp apparatus and are uncorrected.The IR spectra were recorded as KBr pellets on PERKIN-Elmer 1000 units, instruments. All ^{1}H and ^{13}C-NMR spectra were recorded on a Varian XL-300 spectrometer operating at 400MHz for ^{1}H-NMR and 75MHz for ^{13}C-NMR respectively. ^{31}P-NMR spectrum was recorded on a Varian XL-spectrometer operating at 161.89MHz. The compounds were dissolved in DMSO-d_6 and chemical shifts were referenced to TMS (^{1}H and ^{13}C-NMR) and 85% H_3PO_4 (^{31}P-NMR). Elemental analysis was recorded on a Carlo Erba 1108 elemental Analyzer, Central Drug Research Institute, Lucknow, India.

Synthesis of ethyl 2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoro methyl)-4,5-dihydro-1H-pyrazol-1-yl)acetate (3).

The mixture of ethyl 2-(4-(((3,4-dimethoxyphenyl)imino)methyl)-5-oxo-3-(trifluoro methyl)-4,5-dihydro-1H-pyrazol-1-yl)acetate (1,0.02moles,8gms) and PCl_5 (0.013moles, 0.8gms) was heated at 100℃ for 1hr. when the evolution of fumes of HCl ceased, an excess of PCl_5 was removed under reduced pressure and the residual imidoyl chloride (2,0.025moles,10gms) was treated with an ice-cold solution of sodium azide (0.012moles,0.9gms). To the reaction mixture excess of aqueous sodium acetate and acetone was added with stirring. Stirring was continued for overnight. The progress of the reaction was monitored by TLC using cyclohexane and ethyl acetate (7:3) solvent mixture as eluent. After completion of the reaction, the solvent acetone was removed under reduced pressure. The remaining aqueous portion was extracted with chloroform. The chloroform extract was dried under reduced pressure and the residue was purified by column chromatography using silica, cyclohexane and ethyl acetate solvent mixture (7:3) as an eluent. The residue (3, 0.015moles, 6.6gms) was obtained. The resulted solid was identified as ethyl 2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoro methyl)-4,5-dihydro-1H-pyrazol-1-yl)acetate(3).

The structure of ethyl 2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoro methyl)-4,5-dihydro-1H-pyrazol-1-yl)acetate **(3)** was established by spectral analysis (IR and ^{1}H NMR) and elemental analysis.

Yield (75.00%). IR (KBr pellet), ν, cm^{-1}: 3040 cm^{-1}(Ar-H str), 2940 cm^{-1}(CH_2,CH_3 aliphatic CH stretching), 1710 cm^{-1}(>C=O of ester group), 1657cm^{-1}(>C=O str of Pyrazoline-5-one),1500,1430,1375cm^{-1}(str of characteristic of pyrazol-5-one ring), 1340 cm^{-1}(C-F str bond of CF_3), 1240 cm^{-1}(Ar-O-CH_3 str), 1150 cm^{-1}(C-O streching of ester group), 1105,1138,1285, cm^{-1} for –N-N=N- of tetrazole ring. ^{1}HNMR (DMSO-d_6), δ, ppm 1.25 (t,3H.J=12Hz,-CH_3 of ethyl group), 2.20(s,1H,J=7.50,-CH of Pyrazoline-5-one ring), 3.40 (s,6H,two –OCH_3 groups), 4.10 (q,2H,J=12Hz,-OCH_2 of ethyl group), 4.80 (s,2HN-CH_2-C=O) attached to Pyrazoline-5-one ring), 6.80-7.10 (m,3H benzene ring attached to tetrazole ring)., mp 124-126^{o}C. The elemental analysis of $C_{17}H_{17}F_3N_6O_5$ found %C-45.17;%H-3.76;%N-18.22 agreed well with the calculated %C-46.11;%H-3.84;%N-18.98.

Synthesis of 2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoromethyl)-4,5-di hydro-1H-pyrazol-1-yl) acetic acid (4)

A solution of one equivalent ethyl 2-(4-(1-(3,4-dimethoxy phenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetate(3,0.02moles,8.8gms) in solvent mixture tetrahydrofuran/MeOH/H_2O, aqueous NaOH was added and reflux for 6h. The progress of the reaction was

monitored by TLC with ethyl acetate: acetone (4:6) as eluent. After completion, the solvent was evaporated under vacuum to give a crude residue. The residue was washed with ethyl acetate to remove impurities. The residue was acidified with 1N HCl to P^H-2 to give a solid suspension, which was filtered under vacuum to give crude solid. The crude was purified by column chromatography (60-120 mesh-silica gel, eluent:70% ethyl acetate per ether) to afford acid compound 2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoromethyl)-4,5-di hydro-1H-pyrazol-1-yl) acetic acid (4).(4,0.013moles,5.3gms).

Yield (65.00%). IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1}(Ar—H str), 1680cm^{-1}(str. >C=O of carboxylic group),3000cm^{-1}(str. -OH of COOH), 2940cm^{-1}(str. of $–CH_2$ group), 1657 cm^{-1} (>C=O str of Pyrazoline-5-one),1500,1430,1375 cm^{-1}(str. of characteristic of pyrazol-5-one ring), 1340cm^{-1}(C-F str bond of CF_3), 1240cm^{-1}(Ar-O-CH_3 str), 1285,1138,1105 cm^{-1} (–N-N=N- of tetrazole ring),2940cm^{-1} (str. of CH_2, aliphatic). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s,1H,-CH of Pyrazoline-5-one ring), 3.40(s,6H,two –OCH_3 groups),4.80(N-CH_2-C=O,s,2H,-CH_2 attached to Pyrazoline-5-one ring), 4.5(s,1H,OH group of COOH),6.80-7.10(m,3H,benzene ring attached to tetrazole ring), mp 140-142°C. The elemental analysis of $C_{15}H_{13}F_3N_6O_5$ found %C-42.44;%H-3.09;%N-19.87 agreed well with the calculated %C-43.44;%H-3.13;%N-20.27.

Synthesis 1-(benzo[d]oxazol/thiazole/imidazole/-2-yl-methyl)-4-(1-(3,4-dimethoxy phenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (6a-6c)

A mixture of 2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-1-yl) acetic acid (4,0.02moles,8.2gms)) and 2-aminophenol (5, 0.015moles, 1.63gms) were dissolved in sufficient quantity of polyphosphoric acid(PPA). The mixture was heated slowly to 170℃ for 6h, the reaction was monitored by TLC, after completion of the reaction, the reaction mixture was cooled at RT and quenched with an excess of 10% Na_2CO_3 solution and extracted with ethyl acetate. The combined organic layers were washed with water, brine and dried over anhydrous Na_2SO_4 filtered and evaporated to dryness. Purification of the residue by flash chromatography to afford pure 1-(benzo[d]oxazol-2-yl-methyl)-4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one(6a,0.019moles,9.2gms) off-white solid(6a).

The similar synthetic procedure was adopted to synthesize 6b & 6c by employing O-aminothiophenol & O-phenylenediamine respectively.

6a:-Yield (74.00%). IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1} (Ar-H str),3040cm^{-1},1455cm^{-1},1366cm^{-1} & 1270cm^{-1} (str. characterstic bands of benzaoxazole),1657cm^{-1} (>C=O str. Pyrazoline-5-one),1500,1430,1375cm^{-1} (str. charact

eristics of pyrazol-5-one ring),1340cm^{-1} (C-F str band of CF_3), 1240cm^{-1}(Ar-OCH_3 str),1285, 1138,1105cm^{-1} (–N-N=N- of tetrazol ring), 2940cm^{-1}(str. of CH_2 aliphatic). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 3.40(s, 6H, two -OCH_3 groups), 4.8(s, 2H, N-CH_2-C), 6.9-7.5(m, 7H of C_6H_3, C_6H_4 rings), mp 225-227ºC. The elemental analysis of $C_{21}H_{16}F_3N_7O_4$ found %C-51.21; %H-3.19; %N-19.49 agreed well with the calculated %C-51.74; %H-3.28; %N-20.12.

6b:-Yield (70.00%). IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1}(Ar-H str),3040cm^{-1},1500cm^{-1},1000cm^{-1},500cm^{-1}(characteristics of benza thiazole), 1657cm^{-1}(>C=O str. Pyrazoline-5-one),1500,1430,1375cm^{-1} (str. characteristics of pyrazol-5-one ring),1340cm^{-1} (C-F str band of CF_3),1240cm^{-1} (Ar-OCH_3 str), 1285cm^{-1}, 1138cm^{-1}, 1105cm^{-1}(–N-N=N- of tetrazol ring), 2940cm^{-1}(str. of CH_2 alipatic). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 3.40(s, 6H two –OCH_3 groups), 4.8(s, 2H, N-CH_2-C), 6.9-7.3(m, 7H of C_6H_3, C_6H_4 rings), mp 176-178ºC. The elemental analysis of $C_{21}H_{16}F_3N_7O_3S$ found %C-49.00; %H-3.10; %N-18.89 agreed well with the calculated %C-50.09; %H-3.18; %N-19.48.

6c:-Yield (65.00%). IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1} (Ar-H str), 3040cm^{-1},1390cm^{-1} & 1370cm^{-1} (str. characteristics of benzimadazole ring),1657cm^{-1} (>C=O str Pyrazoline-5-one),1500,1430,1375cm^{-1} (str. characteristic of pyrazol-5-one ring),1340cm^{-1} (C-F str band of CF_3),1240cm^{-1} (Ar-OCH_3 str), 1285, 1138, 1105cm^{-1}(–N-N=N- of tetrazol ring), 2940cm^{-1}(str. of CH_2 aliphatic),3420cm^{-1}(str. NH of imidazole ring). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.8(s, 2H, N-CH_2-C), 3.4(s, 6H, two –OCH_3 groups), 6.9-7.2(m, 7H of C_6H_3, C_6H_4 rings),2.4(s,-NH, of imidazole ring), mp 163-165ºC. The elemental analysis of $C_{21}H_{17}F_3N_8O_3$ found %C-51.00; %H-3.39; %N-22.25 agreed well with the calculated %C-51.85; %H-3.49; %N-23.04.

Synthesis of 1-(benzo[d]oxazol/thiazole/imidazole/-2-yl)methyl)-4-(1-(3,4-dihydroxy phenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (7a-c)

A solution of hydroiodicacid (5%) was refluxed in a glass joined apparatus with 1-(benzo[d]oxazol-2-yl-methyl)-4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-3-(trifluoro methyl)-1H-pyrazol-5(4H)-one(6a,0.02moles,9.7gms),1-(benzo[d]oxazol-2-yl) methyl)-4-(1-(3,4-dihydroxyphenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (7a) was liberated from the viscous hydroiodic acid by adding approximately the calculated amount of sodium carbonate solution. After neutralization, the reaction mixture was distilled under reduced pressure to afford crystalised product 1-(benzo[d]oxazol-2-yl)methyl)-4-(1-(3,4-dihydroxyphenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5 (4H)-one(7a,0.014moles,6.4gm)

The similar synthetic procedure was adopted to synthesize 7b & 7c by adopting hydrolysis of 6b and 6c respectively.

7a:Yield (70.00%). IR (KBr pellet), ν, cm^{-1}: 3350cm^{-1} (intra molecular –OH bond), 3040cm^{-1} (Ar-H str.),2940cm^{-1}(aliphatic –CH) 1657cm^{-1} (>C=O str. of Pyrazoline-5-one), 1500,1430, 1375cm^{-1} (str. of characteristics of pyrazol-5-one ring), 1455 & 1366 cm^{-1}(str. characteristics of benzoxazole),1340cm^{-1} (C-F str. band of CF_3), 1285cm^{-1}, 1138cm^{-1},1105cm^{-1}(N-N =N- of tetrazol ring). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 4.6(s, 2H, two -OH groups), 6.9-7.5(m, 7H of

C_6H_3, C_6H_4 rings), mp 184-186°C. The elemental analysis of $C_{19}H_{12}F_3N_7O_4$ found %C-48.56;%H-2.56;%N-20.46 agreed well with the calculated %C-49.67;%H-2.61;%N-21.30.

7b:Yield (65.00%). IR (KBr pellet), ν, cm^{-1}: 3350cm^{-1}(intramolecular –OH bond), 3040cm^{-1}(Ar-Hstr),3030,1500,1000,500cm^{-1} (str. characteristics of benzathiazole), 2940cm^{-1}(aliphatic –C-H-), 1657cm^{-1}(>C=O str of Pyrazoline-5-one),1500,1430,1375cm^{-1}(str. of characteristic of pyrazol-5-one ring), 1340cm^{-1} (C-F str. band of CF_3), 1285cm^{-1}, 1138cm^{-1},1105cm^{-1}(N-N=N- of tetrazol ring). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 4.6(s, 2H two –OH groups), 6.9-7.4(m, 7H of C_6H_3, C_6H_4 rings), mp 152-154°C. The elemental analysis of $C_{19}H_{12}F_3N_7O_3S$ found %C-47.12;%H-2.46;%N-19.87 agreed well with the calculated %C-48.00;%H-2.52;%N-20.63.

7c:Yield (62.00%). IR (KBr pellet), ν, cm^{-1}: 3350cm^{-1} (intra molecular –OH bond), 3040cm^{-1} (Ar-H str.), 3030,1390,1370cm^{-1} (str. characteristics of benzimadazole ring), 2940cm^{-1}(aliphatic –CH), 1657cm^{-1} (>C=O str. of Pyrazoline-5-one), 1500,1430, 1375cm^{-1} (str. characteristics of pyrazol-5-one ring), 1340cm^{-1} (C-F str. band of CF_3), 1285cm^{-1}, 1138cm^{-1},1105cm^{-1}(N-N= N- of tetrazol ring), 3450cm^{-1}(str. –NH of imidazole ring). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(S, 2H, N-CH_2-C), 4.6 (S, 2H, two –OH groups), 7.0-7.3(m, 7H of C_6H_3, C_6H_4 rings), 2.4(s,-NH of imidazole ring). mp 147-149°C. The elemental analysis of $C_{19}H_{13}F_3N_8O_3$ found %C-49.12;%H-2.75;%N-23.62 agreed well with the calculated %C-49.78;%H-2.83;%N-24.45.

Synthesis of 1-(benzo[d]oxazol/thiazole/imidazole/-2-yl)methyl)-4-(1-(2-oxido-2-(4-substituted) phenoxybenzo[d][1,3,2]dioxaphosphol-5-yl)-1h-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (9a-h)

A solution of phenylphosphoricdichloridate (8a, 0.015moles, 3.1gms) in 25ml of dry toluene was added dropwise over a period of 20mits to a stirred solution (7a, 0.02moles, 9.1gms) and triethylamine in 30ml of dry toluene and 10ml of tetrahydrofuran at 5℃, after completion of the addition, the temperature of the reaction mixture was slowly raised to room temperature and stirred for 24hrs. The reaction mixture was later heated to 50-60℃ and maintained for 4hrs with stirring. The completion of the reaction was maintained by TLC analysis. Triethylamine hydrochloric acid was filtered from mixture and solvent was removed under reduced pressure. The residue was washed with water and then recrystallised from aqueous 2-propanol to get pure compound of 1-(benzo[d]oxazol-2-yl)methyl)-4-(1-(2-oxido-2-phenoxybenzo[d][1,3,2]dioxaphosphol-5-yl)-1H-tetrazol5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (9a,0.014moles,8.3gms) with yield of 65% & m.p-128-130℃ The elemental analysis of 9a ($C_{25}H_{15}F_3N_7O_6P$) found %C-49.35;%H-2.44;%N-15.90 agreed well with the calculated %C-50.25;%H-2.51;%N-16.41.

The similar procedure was adopted to synthesize (9a-h) by condensationbetween (7a-c) with dichloro(4-substitutedphenoxy)phosphine (8a-f).

Physical,analytical and spectral data for the analogues (9a-h)

9a: Yield: 65.00%; IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1}(Ar-H str.),1657cm^{-1}(>C=O str. Pyrazoline-5-one), 1615cm^{-1} (str. of >C=N), 1500,1430,1375cm^{-1} (str. characteristics of pyrazoline-5-one ring), 1340cm^{-1} (C-F str.

band of CF_3), 1255cm^{-1}(P=O str. vibrations), 1196cm^{-1} (C_{Aro}-O stretching), 1285, 1138, 1105cm^{-1}(–N-N=N- of tetrazol ring),954cm^{-1} (P-O str. vibrations), 3030,1455,1366cm^{-1} (str. characteristics of benzoxazole ring) and 2940cm^{-1} (str. of CH aliphatic).^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 6.9-7.4 (m, 12H of C_6H_5, C_6H_3,C_6H_4). ^{13}C-NMR (75MH_Z) (DMSO-d_6),δ,ppm: 155.6,24.3,172.1,159.3,122.5,111.3,145.2,145.2,

145.2,117.3,115.3,150.2,120.3,130.1,121.3,130.1,120.3,51.8,152.6,141.5,150.0,110.6,123.8,124.8,

119.1,119.6,and the signals are ascribed as 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_{17}, C_{18},C_{19},C_{20},C_{21},C_{22},C_{23},C_{24},C_{25} carbon atoms respectively.^{31}P-NMR(δ, ppm): -7.3; Mass: 598 (M+1), mp 128-130^{o}C. Elemental Analysis found for $C_{30}H_{23}F_3N_5O_8PS$ is C: 49.35, H: 2.44, N: 15.90.

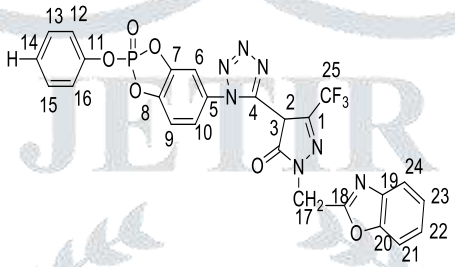


Fig-1:- Structure of 9a.

9b: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3025,1450,1361cm^{-1} (str. characteristics of benzoxazole ring),1495,1425,1370cm^{-1} (str. characteristics of pyrazoline-5-one ring), 1280, 1133, 1100cm^{-1}(–N-N=N- of tetrazol ring), 1250cm^{-1}(P=O str. vibrations), 949cm^{-1} (P-O str. vibrations),1191cm^{-1} (C_{Aro}-O stretching), ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 3.10(s,3H, Ar-CH_3),6.9-7.2 (m, 11H of C_6H_4, C_6H_3,C_6H_4) ^{31}P-NMR(δ, ppm): -6.9; Mass: 612(M+1), mp 112-114^{o}C. Elemental Analysis found for $C_{26}H_{17}F_3N_7O_6P$ is C: 51.16, H: 2.69, N: 15.63.

9c: Yield: 55.00%; IR (KBr pellet), ν, cm^{-1}: 3022,1448,1358cm^{-1} (str. characteristics of benzoxazole ring),1492,1422,1367cm^{-1} (str. characteristics of pyrazoline-5-one ring), 1277, 1130, 1097cm^{-1}(–N-N=N- of tetrazol ring), 1247cm^{-1}(P=O str. vibrations), 946cm^{-1} (P-O str. vibrations),1189cm^{-1} (C_{Aro}-O stretching); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 3.4(s,3H,Ar-OCH_3),6.8-7.1 (m, 11H of C_6H_4, C_6H_3,C_6H_4); ^{31}P-NMR(δ, ppm): -7.2; Mass: 628 (M+1), mp 108-110^{o}C. Elemental Analysis found for $C_{26}H_{17}F_3N_7O_7P$ is C: 48.86, H: 2.65, N: 15.20.

9d: Yield: 64.00%; IR (KBr pellet), ν, cm^{-1}: 3035,1460,1371cm^{-1} (str. characteristics of benzoxazole ring),1505,1435,1380cm^{-1} (str. characteristics of pyrazoline-5-one ring), 1290, 1142, 1110cm^{-1}(–N-N=N- of tetrazol ring), 1260cm^{-1}(P=O str. vibrations), 959cm^{-1} (P-O str. vibrations),1201cm^{-1} (C_{Aro}-O stretching);

^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 7.0-7.5 (m, 11H of C_6H_4, C_6H_3,C_6H_4); ^{31}P-NMR(δ, ppm): -6.3; Mass: 632 (M+1), mp 136-138^{o}C. Elemental Analysis found for $C_{25}H_{14}ClF_3N_7O_6P$ is C: 46.64, H: 2.16,N: 15.20.

9e: Yield: 58.00%; IR (KBr pellet), ν, cm^{-1}: 3033,1458,1369cm^{-1} (str. characteristics of benzoxazole ring),1503,1433,1378cm^{-1} (str. characteristics of pyrazoline-5-one ring), 1288, 1141, 1108cm^{-1}(–N-N=N- of tetrazol ring), 1258cm^{-1}(P=O str. vibrations), 957cm^{-1} (P-O str. vibrations),1199cm^{-1} (C_{Aro}-O stretching); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 7.0-7.4 (m, 11H of C_6H_4, C_6H_3,C_6H_4); ^{31}P-NMR(δ, ppm): -6.7; Mass: 677(M+1), mp 141-143^{o}C. Elemental Analysis found for $C_{25}H_{14}BrF_3N_7O_6P$ is C: 43.54, H: 2.02, N: 14.20.

9f: Yield: 67.00%; IR (KBr pellet), ν, cm^{-1}: 3038,1468,1374cm^{-1} (str. characteristics of benzoxazole ring),1508,1438,1383cm^{-1} (str. characteristics of pyrazoline-5-one ring), 1293, 1146, 1113cm^{-1}(–N-N=N- of tetrazol ring), 1263cm^{-1}(P=O str. vibrations), 963cm^{-1} (P-O str. vibrations),1204cm^{-1} (C_{Aro}-O stretching); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): δ_{ppm}: 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 7.1-7.5 (m, 11H of C_6H_4, C_6H_3,C_6H_4); ^{31}P-NMR(δ, ppm): -6.4; Mass: 666 (M+1), mp 121-123^{o}C. Elemental Analysis found for $C_{26}H_{14}F_6N_7O_6P$ is C: 46.01, H: 2.05, N: 14.33.

9g: Yield: 62.00%; IR (KBr pellet), ν, cm^{-1}: 3016,1442,1352cm^{-1} (str. characteristics of benzoxazole ring),1487,1416,1360cm^{-1} (str. characteristics of pyrazoline-5-one ring), 1270, 1121, 1090cm^{-1}(–N-N=N- of tetrazol ring), 1238cm^{-1}(P=O str. vibrations), 935cm^{-1} (P-O str. vibrations),1165cm^{-1} (C_{Aro}-O stretching); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): δ_{ppm}: 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 6.9-7.4(m, 11H of C_6H_4, C_6H_3,C_6H_4); ^{31}P-NMR(δ, ppm): -7.3; Mass: 614 (M+1), mp 147-149^{o}C. Elemental Analysis found for $C_{25}H_{15}F_3N_7O_5PS$ is C: 48.03, H: 2.40, N: 15.50.

9h: Yield: 65.00%; IR (KBr pellet), ν, cm^{-1}: 3020,1445,1355cm^{-1} (str. characteristics of benzoxazole ring),1490,1420,1365cm^{-1} (str. characteristics of pyrazoline-5-one ring), 1275, 1125, 1095cm^{-1}(–N-N=N- of tetrazol ring), 1243cm^{-1}(P=O str. vibrations), 940cm^{-1} (P-O str. vibrations),1175cm^{-1} (C_{Aro}-O stretching); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): δ_{ppm}: 2.20(s, 1H, -CH of Pyrazoline-5-one ring), 4.3(s, 2H, N-CH_2-C), 6.9-7.4 (m, 11H of C_6H_4, C_6H_3,C_6H_4), 9.4(s,1H, -NH of benzamidazole; ^{31}P-NMR(δ, ppm): -7.3; Mass: 597 (M+1), mp 125-127^{o}C. Elemental Analysis found for $C_{25}H_{16}F_3N_8O_5P$ is C: 49.43, H: 2.61, N: 18.23.

RESULTS AND DISCUSSION:

The synthetic route followed for the synthesis of 2-(4-substituted) phenoxy-1,3,2-benzodioxaphosphole-2-oxide derivatives containing 1,2,3,4-tetrazol-benzoxozole is presented in scheme-1.

Ethyl 2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoro methyl)-4,5-dihydro-1H-pyrazol-1-yl)acetate (3) was prepared by reacting ethyl 2-(4-(((3,4-dimethoxyphenyl)imino)methyl)-5-oxo-3-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetate (1) with $POCl_3$ results imidoyl chloride which on further reaction with sodium azide.

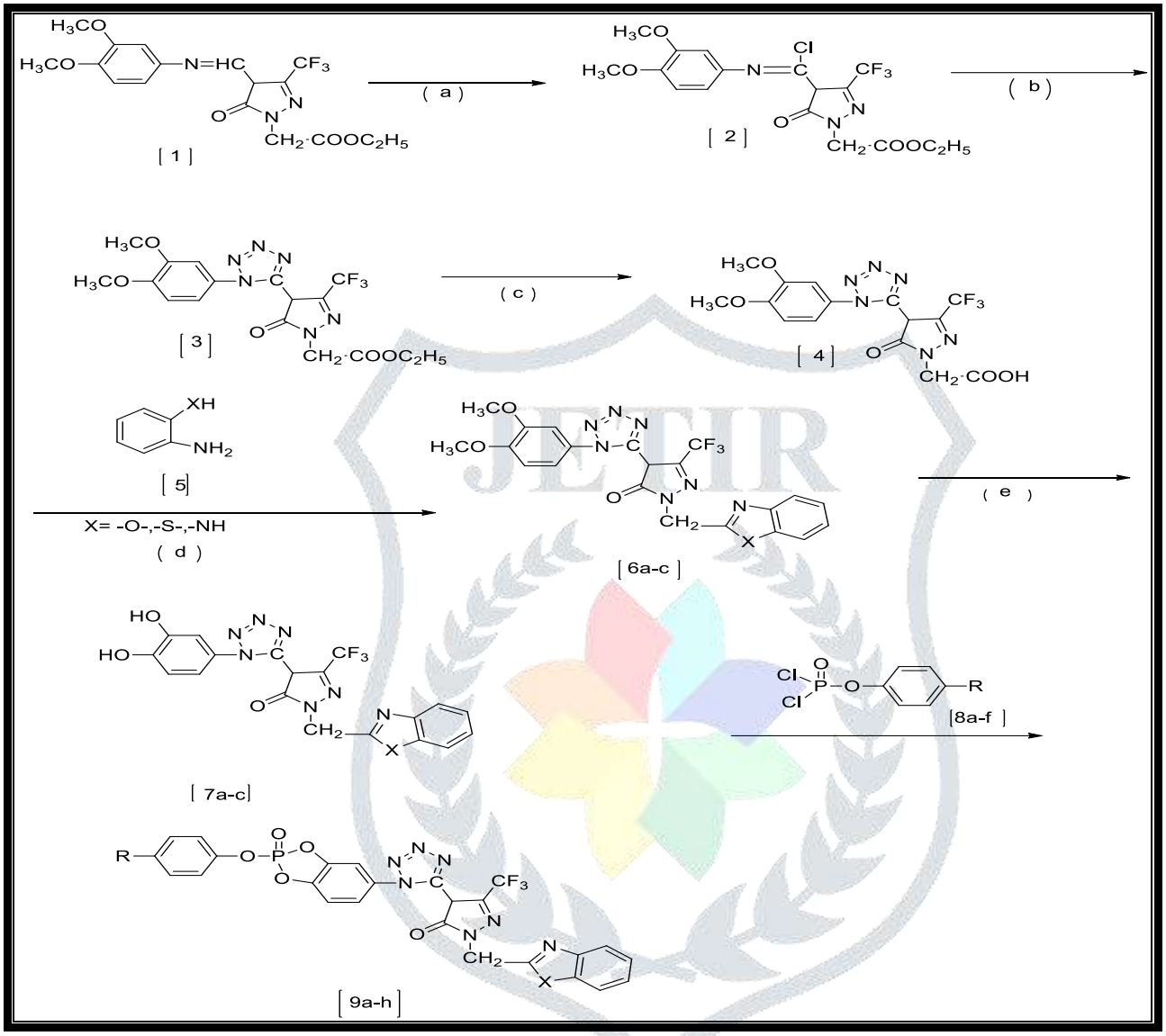
2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoromethyl)-4,5-di hydro-1H-pyrazol-1-yl) acetic acid (4) was prepared by hydrolysis of ethyl 2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetate (3) in solvent mixture tetrahydro furan/MeOH/H_2O, aqueous NaOH refluxed for 6h. Further the 2-(4-(1-(3,4-dimethoxyphenyl)-1H-tetrazol-5-yl)-5-oxo-3-(trifluoromethyl)-4,5-di hydro-1H-pyrazol-1-yl) acetic acid (4) reacts with 2-aminophenol/ o-aminothiophenol/ o-phenylenediamine derivatives(5a-c) in of polyphosphoric acid(PPA) affords 1-(benzo[d]/oxazol/thiazol/imidazol/-2-yl-methyl)-4-(1-(3,4-dimethoxy phenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-ones (6a-6c).

1-(benzo[d]oxazol/thiazol/imidazole/-2-yl)methyl)-4-(1-(3,4-dihydroxy phenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (7a-c) was synthesized by hydrolysis of 1-(benzo[d]/oxazol/thiazol/imidazol/-2-yl-methyl)-4-(1-(3,4-dimethoxy phenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-ones (6a-6c) using hydroiodic acid. The IR spectra of 1-(benzo[d]oxazol/thiazol/imidazole/-2-yl)methyl)-4-(1-(3,4-dihydroxy phenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (7a-c) exhibited bands around 3350cm^{-1} (intra molecular –OH bond). ^1H NMR showed one singlet at δ 4.6(s, 2H, two -OH groups) confirming the structure of 1-(benzo[d]oxazol/thiazol/imidazole/-2-yl)methyl)-4-(1-(3,4-dihydroxy phenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (7a-c).

1-(benzo)[d]oxazol-2-yl-methyl)-4-(1-(2-oxido-2-(4-substi tuted)benzo[d][1,3,2]dioxaphosphol-5-yl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one(9a) was synthesized by reacting 1-(benzo[d]oxazol-2-yl)methyl)-4-(1-(3,4-dihydroxy phenyl)-1H-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (7a) with phenylphosphoricdichloridate(8a) presence of tri ethyl amine as base and dry toluene, THF mixture as solvent at 50-60°C. The IR spectra of 1-(benzo[d]oxazol-2-yl)methyl)-4-(1-(2-oxido-2-phenoxybenzo[d][1,3,2]dioxaphosphol-5-yl)-1H-tetrazol-5-yl)-3-(tri fluoromethyl)-1H-pyrazol-5(4H)-one (9a) exhibited bands around 1255cm^{-1}(P=O str. vibrations), 1196cm^{-1} (C_{Aro}-O stretching), 954cm^{-1} (P-O str.

vibrations). ^{1}H NMR showed multiplet at δ 6.9-7.4 (m, 12H of C_6H_5, C_6H_3,C_6H_4) confirming the structure of 1-(benzo[d]oxazol-2-yl)methyl)-4-(1-(2-oxido-2-phenoxybenzo [d][1,3,2]dioxaphosphol-5-yl)-1H-tetrazol-5-yl)-3-(tri fluoromethyl)-1H-pyrazol-5(4H)-one (9a).

Similarly remaining analogues (9b-h) were prepared.



Scheme-1:-Synthetic route for the preparation of (9a-h).

Reaction conditions:- a) PCl_5,100℃, b) NaN_3 c) Hydrolysis d) Polyphosphoric acid (PPA),170℃,6hrs e) Hydrolysis

Biological activity: The antimicrobial activity of newly synthesized compounds was performed according to disc diffusion method, as recommended by the National Committee for Clinical Laboratory. The synthesized compounds were used at the concentration of 250µg/ml DMSO as a solvent.

Antibacterial activity: The antibacterial activity of 1-(benzo[d]oxazol-2-yl methyl)-4-(1-(2-oxido-2-(4-substituted phenoxy)[d][1,3,2]dioxaphosphol-5-yl)-1h-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5 (4H)-one (9a-h) were screened against the straphylococcus aures NCCS 2079(SA), Bacillus cereus NCCS 2106(BC)(gram+ve) and Escherichia coli NCCS 2065(EC), pseudomonas aeruginosa NCCS 2200(PA)(gram negative) organisms. Most of the compounds exhibit good antibacterial activity against both bacteria.The

antibacterial activity of **(9a-h)** was shown in the **Table-1.** The presence of trifluoromethyl (-CF_3,9f), chloro(-Cl,9d), bromo(-Br,9c) showed more activity than other substituted compounds

The synthesized compounds were used at the concentration of 250 µg/ml using DMSO as a solvent. The amoxycillin was used as a standard. (Hi-media laboratories limited, Mumbai).

Table-1: Antibacterial activity (Diameter zone of inhibition in mm) of Compounds (9a-h) (250µg/ml)

S no	Compound		Zone of inhibition(mm)			
			Antibacterial activity			
			Gram +ve		Gram -ve	
	R	X	SA	BC	EC	PA
9a	-H	-O-	13	11	08	06
9b	-CH_3	-O-	10	08	04	04
9c	-OCH_3	-O-	09	07	06	06
9d	-Cl	-O-	16	15	13	13
9e	-Br	-O-	14	13	11	11
9f	-CF_3	-O-	18	17	15	15
9g	-H	-S-	11	09	06	06
9h	-H	-NH	12	10	07	07
Amoxicillin			22	25	25	27

Antifungal activity: The antifungal activity of 1-(benzo[d]oxazol-2-yl methyl)-4-(1-(2-oxido-2-(4-substitu ted phenoxy)[d][1,3,2]dioxaphosphol-5-yl)-1h-tetrazol-5-yl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (9a-h) were screened against Aspergillus nigaNCCS 1196(AN),Candida albicans NCCS 3471(CA),organisms. Most of the compounds exhibit good antifungal activity against both fungi.

The presence of trifluoromethyl(-CF_3,9f), chloro(-Cl,9d), bromo(-Br,9c) showed more activity than other substituted compounds. Here Ketoconazole was used as reference compound to compare the activity. Here Ketaconazole was used as reference compound to compare the activity.

The antifungal activity of **(9a-h)** was shown in the **Table-2.**

Table-2: Antifungal activity ((Diameter zone of inhibition in mm) of compounds (9a-g)(250µg/ml)

S.No	Comp		Zone of inhibition (mm)	
	R	X	Aspergillius niger NCCS 1196	Candida albicans NCCS 3471
9a	-H	-O-	07	05
9b	-CH_3	-O-	05	03
9c	-OCH_3	-O-	07	05
9d	-Cl	-O-	14	12
9e	-Br	-O-	12	10
9f	-CF_3	-O-	16	14
9g	-H	-S-	07	05

9h	-H	-NH	08	06
Ketoconazole			**22**	**25**

Docking Studies of the compounds (9a-h):

The docking studies of 9a,9b,9c,9d,9e,9f,9g,9h were carried out as model compounds on sortase-A enzyme. The docking ligands were found to have some interactions between an oxygen atom of the ligands and sortase-A enzyme. The results pertaining to Docking studies were shown in the Table **VIII.2.5-6** and fig **VIII.2.13-20.** Moreover, these docked conformations form hydrogen bond interactions with the active site of the enzyme. The common hydrogen bonding interactions were formed between all the docked ligands and THR77:H, ASN163:ND2, TYR54:H, ILE53:N, ILE53:PDB1H, PHE76:O. The order of enzyme-ligand hydrogen bond energy (S(Hb_ext)) is 9b>9g>9h>9a>9c>9e>9f>9d. The vanderwaals interactions between ligand-enzyme were also noticed. The order of enzyme-ligand vanderwaals score of interaction was found to be 9c>9b>9f>9a>9g>9h>9e>9d. However the ligands fail to exhibit intramolecular hydrogen bonding with the enzyme. The ligands exhibit minimum intramolecular strain. Finally, all the ligands exhibit moderate to good antibacterial activity with Sortase-A enzyme. The order of gold score fitness value of the ligands is 9g>9h>9f>9a>9b>9c>9e>9d. According to gold score fitness value ligand 9g exhibits high binding activity with the enzyme and ligand 9d showed leads binding activity with the enzyme.

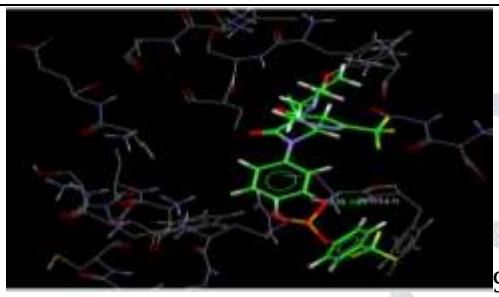
Table-3: Docking results of ligands (9a-h) with Sortase A enzyme.

enzyme.

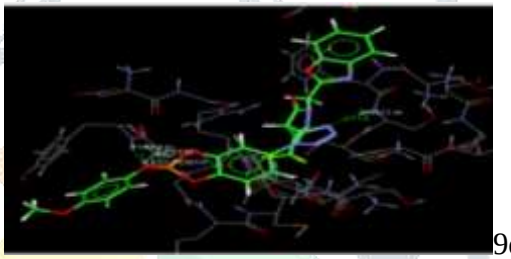
Comp	X	R	Fitness	S(Hb_ex)	S(vdw_et)	S(Hb_in)	S(vdw_it)
9a	O	-H	55.33	3.36	39.19	0.00	-1.93
9b	O	$-CH_3$	55.01	3.91	39.74	0.00	-3.54
9c	O	$-OCH_3$	54.89	3.10	40.03	0.00	-3.25
9d	O	-Cl	50.50	2.62	36.51	0.00	-2.32
9e	O	-Br	51.35	3.05	36.94	0.00	-2.50
9f	O	$-CF_3$	55.70	2.94	39.43	0.00	-1.45
9g	-S-	-H	56.53	3.79	39.17	0.00	-1.12
9h	-NH	-H	56.42	3.77	39.14	0.00	-1.16

Comp	R	X	No of 'H' bonds	Compounds		Bond Length (A°)	Fitness
				Protein	Atoms		
9a	H	O	2	THR77:H TYR54:H	N9 O:22	2.266 1.817	55.33
9b	$-CH_3$	O	2	TYR54:H THR77:H	O:22 N9	1.623 2.209	55.01
9c	$-OCH_3$	O	2	THR77:H TYR54:H	O:22 N9	2.116 1.748	54.89

9d	-Cl	O	1	TYR54:H	O:22	2.027	50.50
9e	-Br	O	3	THR77:H THR77:H TYR54:H	N9 N10 O:22	1.837 2.126 1.822	51.35
9f	$-CF_3$	O	2	THR77:H TYR54:H	N9 O:22	2.522 1.786	55.70
9g	H	-S-	2	THR77:H TYR54:H	N9 O:22	2.456 1.772	56.53
9h	H	-NH	3	PHE76: O THR77:H TYR54:H	H54 N9 O:22,H49	2.290 2.332 1.762	56.42


9a

9b

9c

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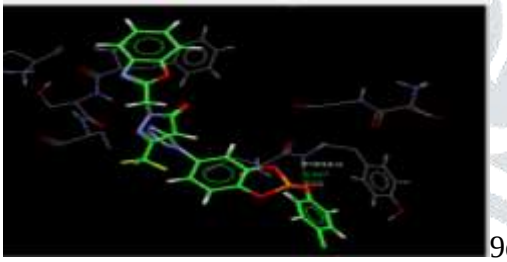
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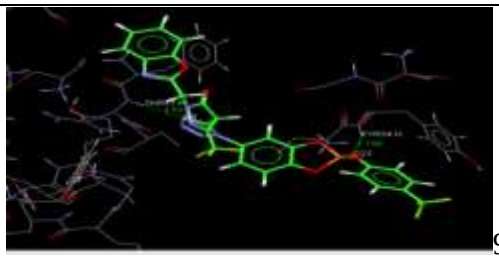
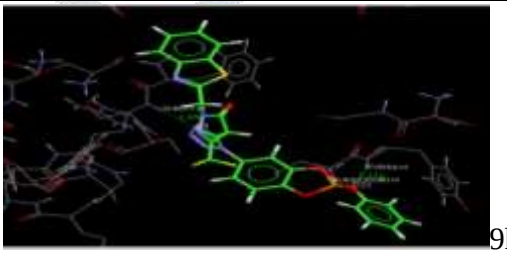
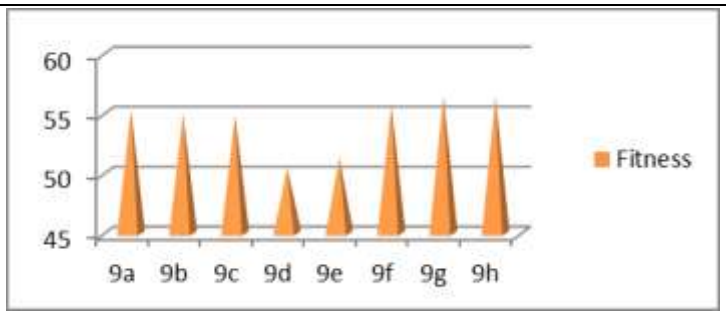
9f

9g

9h

Fig: Ⅷ.2.13-20 docking images of tetrazol-benzoxozoles with Sortase-A



Comparative Gold score fitness values for compounds (9a-h)

The results of docking study of newly synthesized 2-(4-substituted) phenoxy-1,3,2-benzodioxaphosphole-2-oxide derivatives containing 1,2,3,4-tetrazol-benzoxozole reveals that all the compounds are having good interaction in favourable pose of Sortase-A. Among eight 2-(4-substituted) phenoxy-1,3,2-benzodioxaphosphole-2-oxide derivatives containing 1,2,3,4-tetrazol-benzoxozole, four derivatives (9a,9f, 9e &9d) showed better activity.

CONCLUSION:

In current research work, few analogues of 2-(4-substituted) phenoxy-1,3,2-benzodioxaphosphole-2-oxide derivatives containing 1,2,3,4-tetrazol-benzoxozole were successfully prepared and characterized. Biological activity and docking studies of these compounds were also studied. Among eight analogues four derivatives shown better activity and these can be taken as lead compounds for further development in future.

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