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SYNTHESIS, CHARACTERISATION, DOCKING STUDIES AND ANTIMICROBIAL EVALUATION OF ETHYL 3-(5-(2-OXIDO-2-(4-SUBSTITUTED PHENOXY) BENZO[*D*][1,3,2]DIOXAPHOS PHOL-5-YL)-1*H*-TETRAZOL-1-YL)THIOPHENE-2-CARBOXYLATES

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ABSTRACT

The reaction of ethyl 3-((3,4-dimethoxybenzylidene)amino) thiophene-2-carboxylate (5) reacts with phosphorus tri chloride followed by sodium azide in presence znic chloride, sodium acetae in tetrahydrofuran forms ethyl 3-(5-(3,4-dimethoxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (6) which on hydrolysis affords the ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (7). A new series of ethyl3-(5-(2-oxido-2-(4-substituted phenoxy)benzo[*d*][1,3,2]dioxaphosphol-5-yl)-1*H*-tetrazol-1-yl)thiophene-2-carboxylates (9a-g) were synthesized from ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate **(7)** by condensing with 4-substituted phenyl phosphoro dichloridates (8a-g). The structures of these analogues (9a-g) have been established by ^{1}H NMR, IR, Mass spectral data and elemental analysis. This study describes the anti-microbial activity and docking studies of newly synthesized analogues (9a-g).

KEYWORDS: Ethyl-2-cyanoacetate, 2,5-dihydroxy-1,4-dithiane, 3,4-dimethoxy benzaldehyde, sodium azide, antimicrobial activity and docking studies.

INTRODUCTION

Tetrazoles and its derivatives are associated with a variety of biological activities such as antifungal[1], antinociceptive[2-3], anti convulsant[4], antidiabetic[5], cyclo-oxygenase inhibitors[6], hypoglycaemic[7], antibacterial[8] and anti-inflammatory[9] activities. Tetrazoles are used as catalysts in the synthesis of phosphonates. In the present studies we have developed a molecular frame, which consists of both organo-phosphorus and tetrazol moieties. Thus different ethyl 3-(5-(2-oxido-2-(4-substituted phenoxy) benzo[*d*][1,3,2]dioxaphos phol-5-yl)-1*H*-tetrazol-1-yl)thiophene-2-carboxylates(9a-g) were synthesized. The structures of these analogues have been established by Mass, NMR, IR studies, elemental analysis and synthesis. All the new compounds were screened for their anti-microbial activity. Some of the derivatives found to have promising activity.

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 3-(5-(3,4-dimethoxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (6).

The compound ethyl 3-((3,4-dimethoxybenzylidene) amino)thiophene-2-carboxylate**(5)** was converted to ethyl 3-(5-(3,4-dimethoxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate**(6)** on treatment with PCl_5, sodium azide in Tetrahydrofuran. The reaction mixture was heated for one hour at 100°C. The progress of the reaction was monitored by TLC with n-hexane:ethylacetate(7:3) as an elutent.

The structure of ethyl 3-(5-(3,4-dimethoxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate**(6)** was established by spectral analysis (IR and ^{1}H NMR) and elemental analysis.

Yield (75.00%). IR (KBr pellet), ν, cm^{-1}: 3020-3040cm^{-1}($\gamma_{\text{-Ar-H}}$ of benzene ring and thiophene ring), 2960 cm^{-1};

2890 cm^{-1} (γ_{-CH} of CH_3 and CH_2 groups), 1695 cm^{-1} (stretching of carbonyl group of an ester), 1450 cm^{-1}-675 cm^{-1} (characteristics of thiophene ring), 1415 cm^{-1} (stretching of C-N of tetrazole ring), 1240cm^{-1} (stretching of C-O of an ester), 1050cm^{-1} ($\delta_{-c-o-c-}$ of aromatic ether) and 1157 cm^{-1}, 2120 cm^{-1}due to stretching vibrations of tetrazole and azide respectively. ^{1}HNMR (DMSO-d_6), δ, ppm 1.2(t,3H, of $-CH_3$ group of ester), δ_{ppm} 4.2(q,2H, -CH_2 of an ester), δ_{ppm} 3.80(s,6H,two $-OCH_3$ groups), and 6.9-7.3(m,5H, 3H of C_6H_3 ring and two thiophene protons)., mp 136-138^{o}C.

Synthesis of ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (7)

A solution of ethyl 3-(5-(3,4-dimethoxyphenyl)-1*H*-tetrazol-1-yl)thiophene-2-carboxylate (**6**,0.02moles) was dissolved in 30ml CH_2Cl_2 under N_2 and boron tri bromide (2.4ml, 0.025moles) was added at -78°C. The mixture was warmed slowly to room temperature and stirred for 16 hours. Cold methanol and ice water was added to quench reaction and saturated aqueous $NaHCO_3$ solution was used to adjust P^H to 7~8. After extracting three times by ethyl acetate, each time 25ml, the organic layer was merged and dried by anhydrous Na_2SO_4. It was then purified by column chromatography (elutent Petrolium ether**:** Ethyl acetate 8:2) to give the product ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate(7)**.**

Yield (75.00%). IR (KBr pellet), ν, cm^{-1}: 3350 cm^{-1} (intra molecular hydrogen bonding γ_{-OH}), 3040cm^{-1}(γ_{-Ar-H} of benzene ring and thiophene ring), 2960 cm^{-1}; 2890 cm^{-1} (γ_{-CH} of CH_3 and CH_2 groups), 1695 cm^{-1} (stretching of carbonyl group of an ester), 1450 cm^{-1}, 675cm^{-1} (characteristics of thiophene ring), 1415 cm^{-1}(stretching of C-N of tetrazole ring), 1240cm^{-1} (stretching of C-O of an ester) and 1157 $cm^{-1}$and 2120 cm^{-1} (stretching vibrations of tetrazole and azide respectively). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.2(t,3H, of $-CH_3$ group of ester), δ_{ppm} 4.2(q,2H, -CH_2 of an ester), 6.9-7.3(m,5H, 3H of C_6H_3 ring and two thiophene protons) and δ_{ppm} 5.6 (s,2H, of two –OH groups), mp 184-186^{o}C.

Synthesis of 4-substituted phenyl phosphorodichloridates(8a-g)

4-substituted phenyl phosphoro dichloridates (8a-g) were prepared as per literature procedure.

Synthesis of ethyl 3-(5-(2-oxido-2-phenoxybenzo[*d*]/2-(*p*-tolyloxy)benzo[*d*]/2-(4-fluorophenoxy/4-chlorophenoxy/4-bromophenoxy/4-(trifluoromethyl) phenoxy/4-nitro phenoxy)-2-oxidobenzo[*d*] [1,3,2] dioxaphosphol-5-yl)-1*H*-tetrazol-1-yl)thiophe -ne-2-carboxylate(9a-g)[10]

A solution of phenyl phosphorodichloridate (**8a,**0.025moles) in 25ml of dry toluene was added drop wise over a period of 20 minutes to a stirred solution of ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene -2-carboxylate(**7**,0.02moles) and triethylamine (0.04moles) in 30ml of dry toluene and 10ml of Tetra Hydro Furan at 5°C. After completion of addition, the temperature of the reaction mixture was slowly raised to room temperature and stirred for 2hours. Later the reaction mixture was heated to 50-60°C and maintained for 4hours with stirring. The completion of the reaction was monitored by TLC analysis. Tri ethylamine hydrochloric acid was filtered from mixture and solvent was removed under reduced pressure. The residue was washed with water and then recrystallized from aqueous 2-propanol to get pure compound, ethyl 3-(5-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-1*H*-tetrazol-1-yl)thiophene-2-carboxylate**(9a)**. The m. p. of **(9a)** was found to be 95-97°C with a yield of 60%, 0.012moles. The separated solid was identified as ethyl 3-(5-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-1*H*-tetrazol-1-yl)thiophene-2-carboxylate**(9a)**.

The similar procedure was adopted to synthesize **(9b-g)** by the condensation reaction between ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (**7)** with *p*-tolyl phosphorodichloridate**(8b)**, 4-fluorophenyl phosphorodichloridate**(8c),** 4-chlorophenyl phosphorodichloridate**(8d),** 4-bromophenyl phosphorodichloridate**(8e),** 4-trifluoromethyl phenyl phosphorodichloridate**(8f)** and 4-nitrophenylphosphorodichloridate**(8g).**

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

9a: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1}(γ_{Ar-H} of Benzene ring and thiophene ring), 2960 cm^{-1}; 2890 cm^{-1} (γ_{-CH} of CH_3 and CH_2 groups), 1695 cm^{-1} (γ  of an ester), 1450 cm^{-1}-675 cm^{-1} (characteristics of thiophene ring), 1415cm^{-1} (stretching of C-N of tetrazole ring), 1250cm^{-1}(stretching vibration of P=O), 1050cm^{-1} ($\delta_{-c-o-c-}$ of aromatic ether) and 954cm^{-1}(stretching vibration of P-O-$C_{(-Ar)}$.^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, -CH_3 group of an ester), δ_{ppm} 4.24 (q,2H, -CH_2 of an ester and 6.9-7.3(m,10H, C_6H_3 , $C_6H_5$and 2H of thiophene ring). ^{13}C-NMR (75MH_Z) (DMSO-d_6),δ,ppm: 133.2, 133.8, 128.6, 153.3, 163.5, 124.6, 114.3, 145.7, 145.2, 117.8, 123.3, 150.2, 120.3, 130.1, 121.3,162.3 and 60.9 and 14.1 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_{17}, C_{14}& C_{16}, C_{15} , C_{18}, C_{19} and C_{20} respectively. The molecule has 18- non-equivalent protons. ^{31}P-NMR(δ, ppm): -6.90; Mass: 470(M+1), mp 95-97^{o}C. Elemental Analysis found for $C_{20}H_{15}N_4O_6PS$ is C: 50.56, H: 3.67, N: 11.45, P: 6.24, S: 6.39.

9b: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3025cm^{-1} (Ar-H),2955, 2885 (γ_{-CH} of CH_3&CH_2) , 1695 (C=O), 1133,1280,1100(N-N=N, tetrazole ring),1410 (C-N), 1245(P=O), 945 P-O-C(-Ar); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, -CH_3 group of an ester), δ_{ppm} 2.40(s,3H of methyl group), δ_{ppm} 4.24 (q,2H, -CH_2 of an ester and 6.9-7.3(m,9H, C_6H_3, C_6H_4 and 2H of thiophene ring); ^{31}P-NMR(δ, ppm): -6.8; Mass: 484(M+1), mp 81-

83°C. Elemental Analysis found for $C_{21}H_{17}N_4O_6PS$ is C: 51.56, H: 3.03, N: 11.08, P: 5.92, S: 6.15.

9c: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1} (Ar-H),2970, 2900 (γ_{-CH} of CH_3&CH_2) , 1690 (C=O), 1147,1295,1115(N-N=N, tetrazole ring),1420 (C-N), 1255(P=O), 960 P-O-C(-Ar); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, -CH_3 group of an ester), δ_{ppm} 4.24 (q,2H, -CH_2 of an ester and 7-7.3(m,9H, C_6H_3, C_6H_4 and 2H of thiophene ring); ^{31}P-NMR(δ, ppm): -7.6; Mass: 488 (M+1), mp 89-91°C. Elemental Analysis found for $C_{20}H_{14}FN_4O_6PS$ is C: 48.71, H: 2.47,F: 3.37, N: 11.04, P: 5.90, S: 6.10.

9d: Yield: 65.00%; IR (KBr pellet), ν, cm^{-1}: 3035cm^{-1} (Ar-H),2965, 2895 (γ_{-CH} of CH_3&CH_2) , 1690 (C=O), 1142,1290, 1110(N-N=N, tetrazole ring),1417 (C-N), 1253(P=O), 955 P-O-C(-Ar); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, -CH_3 group of an ester), δ_{ppm} 4.24 (q,2H, -CH_2 of an ester and 7.0-7.3(m,9H, C_6H_3, C_6H_4 and 2H of thiophene ring); ^{31}P-NMR(δ, ppm): -7.3; Mass: 504(M+1), mp 110-112°C. Elemental Analysis found for $C_{20}H_{14}ClN_4O_6PS$ is C: 47.03, H: 2.38 Cl: 6.58, N: 10.68, P: 5.74, S: 5.92.

9e: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3035cm^{-1} (Ar-H),2960, 2890 (γ_{-CH} of CH_3&CH_2) , 1690 (C=O), 1141,1288,1108(N-N=N, tetrazole ring),1417 (C-N), 1253(P=O), 955 P-O-C(-Ar); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, -CH_3 group of an ester), δ_{ppm} 4.24 (q,2H, -CH_2 of an ester and 7-7.3(m,9H, C_6H_3, C_6H_4 and 2H of thiophene ring); ^{31}P-NMR(δ, ppm): -7.3; Mass: 550(M+1), mp 123-125°C. Elemental Analysis found for $C_{20}H_{14}BrN_4O_6PS$ is C: 43.28, H: 2.18, Br: 14.09, N: 9.74, P: 5.22, S: 5.37.

9f: Yield: 70.00%; IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1} (Ar-H),2970, 2900 (γ_{-CH} of CH_3&CH_2) , 1690 (C=O), 1146,1293,1113(N-N=N, tetrazole ring),1420 (C-N), 1260(P=O), 965 P-O-C(-Ar); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): δ_{ppm}: 1.29(t,3H, -CH_3 group of an ester), δ_{ppm} 4.24 (q,2H, -CH_2 of an ester and 7-7.3(m,9H, C_6H_3, C_6H_4 and 2H of thiophene ring); ^{31}P-NMR(δ, ppm): -8.10; Mass: 538(M+1), mp 113-115°C. Elemental Analysis found for $C_{21}H_{14}F_3N_4O_6PS$ is C: 46.37, H: 2.18, F: 10.19 N: 9.93, P: 5.38, S: 5.54.

9g: Yield: 70.00%; IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1} (Ar-H),2975, 2905 (γ_{-CH} of CH_3&CH_2) , 1690 (C=O),1153,1300,1120(N-N=N, tetrazole ring),1425 (C-N), 1270(P=O), 970 P-O-C(-Ar); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, -CH_3 group of an ester), δ_{ppm} 4.24(q,2H, -CH_2 of an ester and 7.1-7.3(m,9H, C_6H_3, C_6H_4 and 2H of thiophene ring).^{31}P-NMR(δ, ppm): -8.8; Mass: 515 (M+1), mp 132-134°C. Elemental Analysis found for $C_{20}H_{14}N_5O_8PS$ is C: 46.22, H: 2.33, N: 13.18, P: 5.63, S: 5.83.

RESULTS AND DISCUSSION

The synthetic route followed for the synthesis of ethyl 3-(5-(2-oxido)-(4-substituted phenoxy)-benzodioxaphosphol-tetrazol-thiophene-2-carboxylates is presented in scheme-1.

Ethyl 3-aminothiophene-2-carboxylate (3) was prepared by reacting ethyl 2-cyanoacetate with 2,5-dihydroxy-1,4-dithiane in presence of catalytic amount of tri ethyl amine in ethanol at reflux temperature.

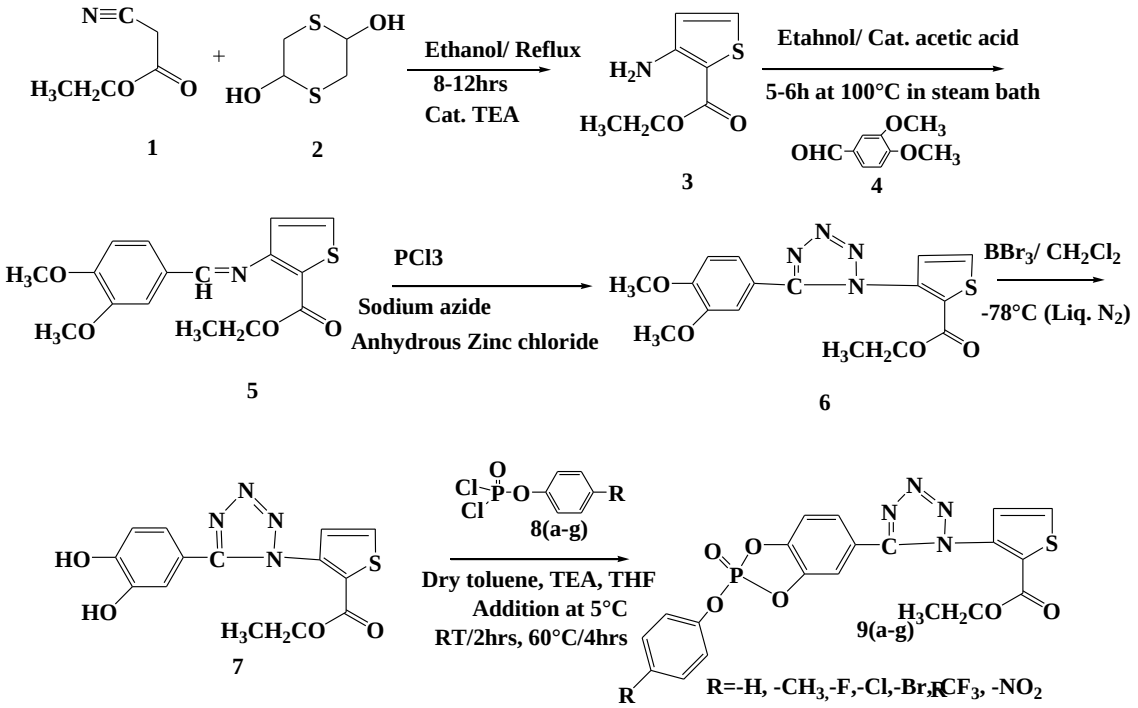
Ethyl 3-((3,4-dimethoxybenzylidene)amino) thiophene-2-carboxylate(5) was prepared by reacting ethyl 3-aminothiophene-2-carboxylate (3) with 3,4-dimethoxy benzaldehyde (4) in presence of few drops of acetic acid at 100°C on a steam bath. Further the Ethyl 3-((3,4-dimethoxybenzylidene)amino) thiophene-2-carboxylate(5) reacts with PCl_3 followed by sodium azide in tetrahydrofuran as a solvent affords ethyl 3-(5-(3,4-dimethoxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (6). The IR spectra of ethyl 3-(5-(3,4-dimethoxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (6) exhibited bands around 1415 cm^{-1} (stretching of C-N of tetrazole ring), 1240cm^{-1} (stretching of C-O of an ester), 1050cm^{-1} ($\delta_{-c-o-c-}$ of aromatic ether) and 1157 cm^{-1}, 2120 cm^{-1}due to stretching vibrations of tetrazole and azide respectively. ^{1}H NMR of (6) showed one singlet at δ 3.80(s,6H,two –OCH_3 groups), and 6.9-7.3(m,5H, 3H of C_6H_3 ring and two thiophene protons) confirming the structure of compound (6). ^{1}H NMR of (6) showed absence of singlet at 8.30(s, H, C-H of azo methine group) which is present in (5) confirming its structure.

Ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (7) was synthesized by hydrolysis of ethyl 3-(5-(3,4-dimethoxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (6) using boran tri bromide. The IR spectra of ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (7) exhibited bands around 3350 cm^{-1} (intra molecular hydrogen bonding γ_{-OH}) 1415 cm^{-1}(stretching of C-N of tetrazole ring), 1240cm^{-1} (stretching of C-O of an ester) and 1157 $cm^{-1}$and 2120 cm^{-1} (stretching vibrations of tetrazole and azide respectively). ^{1}H NMR showed one singlet at δ 5.6 (s,2H, of two –OH groups) confirming the structure of ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (7).

Ethyl 3-(5-(2-oxido-2-(4-substituted phenoxy) benzo[*d*][1,3,2]dioxaphosphol-5-yl)-1*H*-tetrazol-1-yl)thiophene-2-carboxylates (9a-g) were prepared by condensing ethyl 3-(5-(3,4-dihydroxyphenyl)-*1H*–tetrazol-1-yl)thiophene-2-carboxylate (7) with 4-substituted phenyl phosphoro dichloridates (8a-g) in presence of tri ethyl amine as base and dry toluene, THF mixture as solvent at 50-60°C. The IR spectra of ethyl 3-(5-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-1*H*-tetrazol-1-yl)thiophene-2-carboxylate (9a)

exhibited bands around 1695 cm^{-1} (γ —C(=O)— of an ester), 1450 cm^{-1}-675 cm^{-1} (characteristics of thiophene ring), 1415cm^{-1} (stretching of C-N of tetrazole ring), 1250cm^{-1}(stretching vibration of P=O), 1050cm^{-1} ($\delta_{-c\text{-}o\text{-}c-}$ of aromatic ether) and 954cm^{-1}(stretching vibration of P-O-$C_{(-Ar)}$. ^{1}H NMR showed multiplet at δ 6.9-7.3(m,10H,C_6H_3 , C_6H_5 and two thiophene protons) confirming the structure of ethyl 3-(5-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-1*H*-tetrazol-1-yl)thiophene-2-carboxylate (9a)

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



Scheme-1:-Synthetic path way for the preparation of (9a-g)

Biological activity: The antimicrobial activity[11] of newly synthesized compounds was performed according to disc diffusion method, as recommended by the National Committee for Clinical Laboratory.[12] The synthesized compounds were used at the concentration of 250µg/ml. DMF as a solvent.

Antibacterial activity: The antibacterial activity[13] of ethyl 3-(5-(2-oxido)-(4-substituted phenoxy)-benzodioxaphosphol-tetrazole-thiophene-2-carboxylates**(9a-g)** were screened against the Staphylococcus aureus (gram positive), Bacillus cereus, Escherichia coli (gram negative) and Pseudomonas aeruginosa organisms. The compounds with substituents nitro **(9g),** trifluoro methyl **(9f)** and fluoro **(9c)** showed more activity than other substituted compounds. The antibacterial activity of **(9a-g)** was shown in the **Table-1** and **Fig-1.** Here Amoxicillin is used as the reference compound to compare the activity.

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

S.No	Comp	Zone of inhibition (mm)			
		Staphylococcus aureus NCCS 2079	**Bacillus cereus NCCS 2016**	**Escherichia coli NCCS 2065**	**Pseudomonas aeruginosa NCCS 2200**
1	9a	10	5	6	8
2	9b	8	5	6	7
3	9c	14	8	10	11
4	9d	12	7	9	10
5	9e	11	6	7	11
6	9f	15	9	11	13
7	9g	17	12	13	15
Amoxicillin		21	27	24	22

Antifungal activity: Antifungal activity of final compounds ethyl 3-(5-(2-oxido)-(4-substituted phenoxy)-benzodioxaphosphol-tetrazole-thiophene-2-carboxylates**(9a-g)** were screened against Aspergillus niger, Candida albicans. The compounds with substituents nitro **(9g),** trifluoro methyl**(9f)** and fluoro **(9c)** showed more activity than other substituted compounds. The antifungal activity of **(9a-g)** was shown in the **Table-2** and **Fig-2.** Here ***Ketoconazole*** is used as reference compound to compare the activity.

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

S.No	Comp	Zone of inhibition (mm)	
		Aspergillius niger NCCS 1196	Candida albicans NCCS 3471
1	9a	9	7
2	9b	7	6
3	9c	14	12
4	9d	12	10
5	9e	11	9
6	9f	16	13
7	9g	18	15
Ketoconazole		22	25

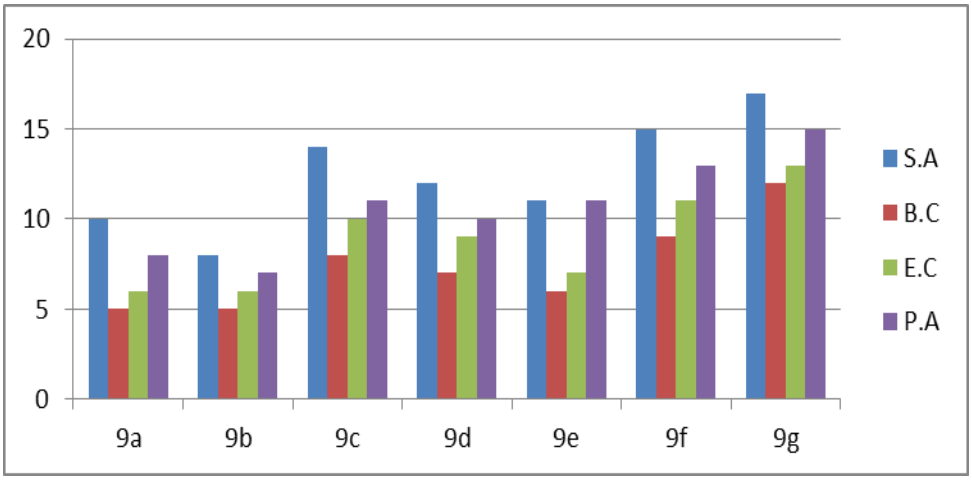


Fig 1: Antibacterial activity of compounds 9(a-g).

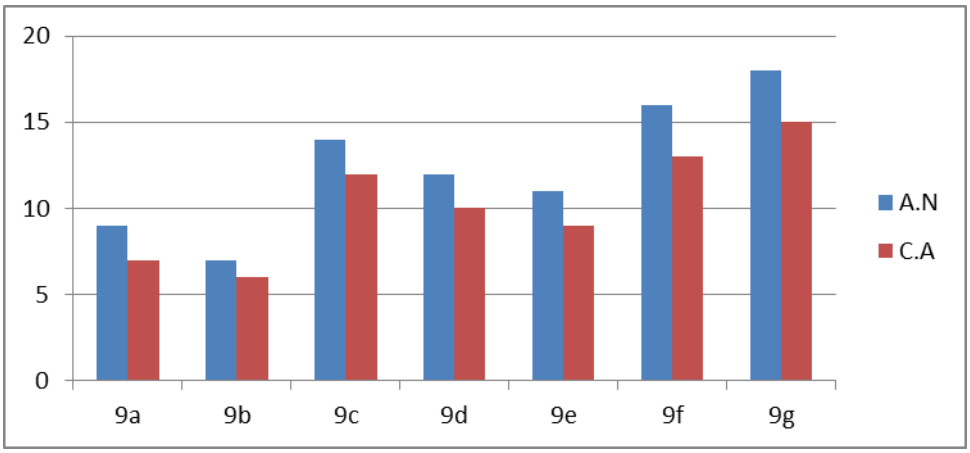
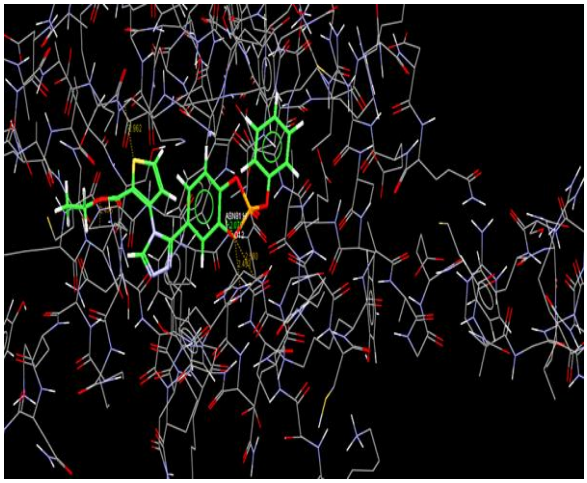


Fig. 2: Antifungal activity of compounds 9(a-g).

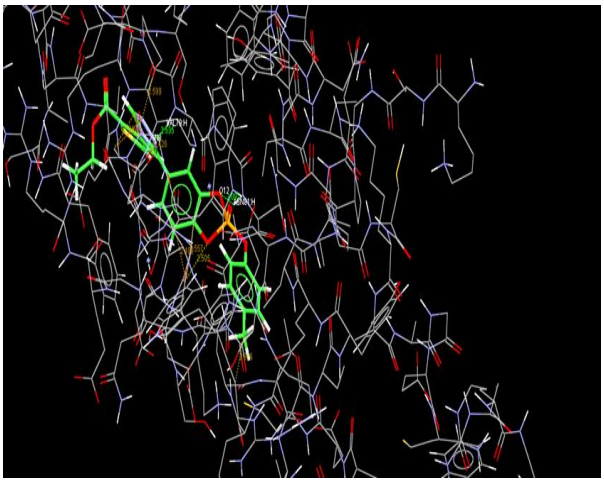
Docking Studies of the compounds (9a-g): Docking[14] of the inhibitors (synthesized compounds) from (**9a-g)** was carried out with Reverse Transcriptase domain using GOLD (Genetic Optimization of Ligand Docking) software 3.0.1, which is based on genetic algorithm (GA). The docking studies of (**9a-g)** were carried out as model compounds on HIV reverse transcriptase.[15] The docking ligands were found to have some interactions between an oxygen atom of the ligands and HIV reverse transcriptase protein. The results pertaining to Docking studies were shown in the **Table-3-Table-4** and in **Fig-4.** Moreover, these docked conformations form hydrogen bond interactions with the active site of the protein. The common hydrogen bonding interactions were formed

between all the docked ligands and amino acid part of the protein. The hydrogen bonding was formed between the amino acid part of the protein and active Oxygen atom of the **(9a-g).** The hydrogen bondings were noticed between Asparagine(81) and Valine (79). The order of protein-ligand vanderwaals score of interaction was found to be **9g>9c>9c>9d>9a>9f>9e** with the protein. However the ligands fail 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 protein. The order of gold score fitness value of the ligands is **9g>9c>9c>9d>9a>9f>9e**. According to gold score fitness value ligand **9g** exhibits high binding activity with the protein and ligand **9e** showed least binding activity with the protein. Comparative Gold Score fitness values for **(9a-g)** were shown in **Fig.4.**

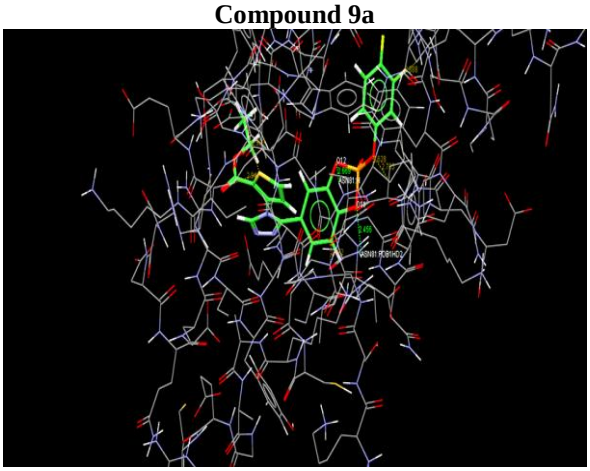
In Gold score evaluation of docking studies, electronic interactions, bonding interactions and steric interaction and conformations of proteins and docked ligand play significant role. However, in the evaluation of antimicrobial studies, electronic factors of the substituents play a significant role.



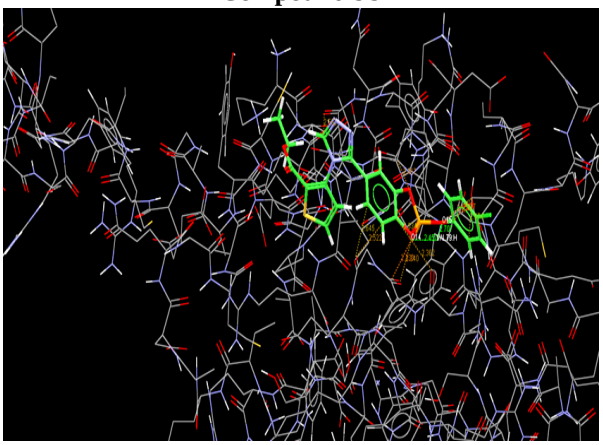
Compound 9a



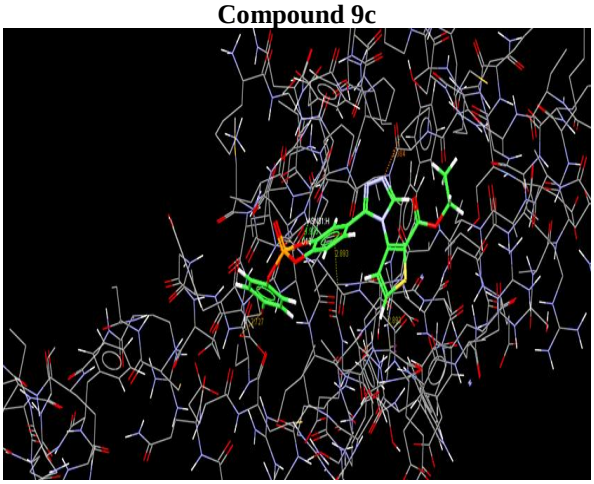
Compound 9b



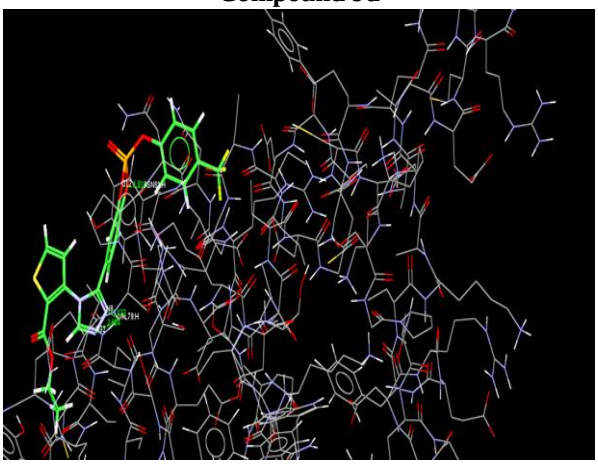
Compound 9c



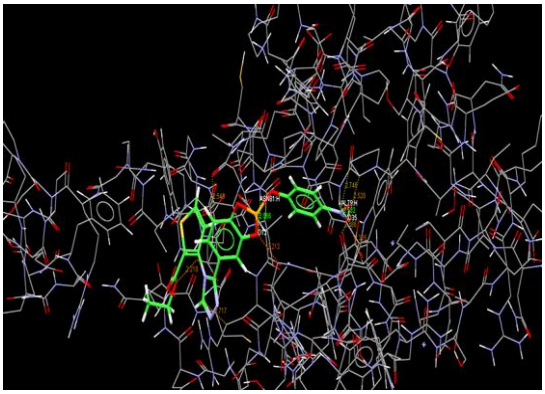
Compound 9d



Compound 9e



Compound 9f



Compound 9g

Fig.3: Docking studies of the compounds (9a-g).

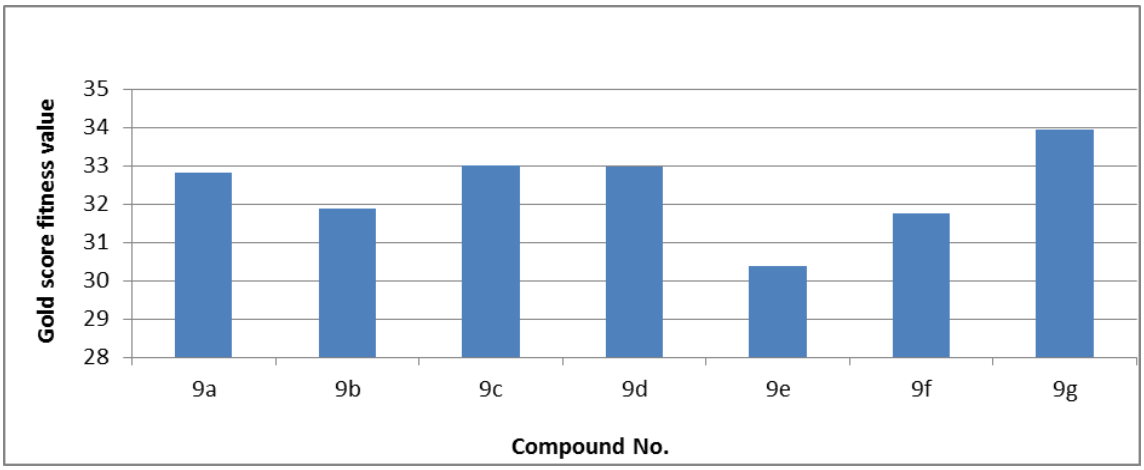


Fig.4.:Comparative Gold Score Fitness values for Compounds(9a-g)

Table-3: Docking results of (9a-g) on Phytase protein.

Comp No.	R	Fitness	S(Hb_ext)	S(vdw_ext)	S(Hb_int)	S(vdw_int)
9a	H	32.82	0.36	37.56	0.00	-18.83
9b	CH_3	31.89	0.00	35.21	0.00	-16.52
9c	F	33.00	0.00	35.41	0.00	-15.68
9d	Cl	32.98	0.00	36.99	0.00	-17.88
9e	Br	30.38	0.00	36.08	0.00	-19.22
9f	CF_3	31.76	0.00	35.89	0.00	-17.59
9g	NO_2	33.95	0.00	37.14	0.00	-17.11

Table-4.: Hydrogen bonding interactions of Compounds (9a-g) with HIV Reverse Transcriptase.

Comp No	R	No of 'H' bonds	Compounds		Bond Length (A^o)	Fitness
			Protein	Atoms		
9a	H	1	ASN81:H	O12	2.079	32.82
9b	CH_3	2	ASN81:H VAL79:H	O12 N8	2.092 2.535	31.89
9c	F	2	ASN81:H ASN81:PDB1HD2	O12 O14	2.669 2.456	33.00
9d	Cl	2	VAL79:H VAL79:H	O15 O14	2.707 2.452	32.98
9e	Br	1	ASN81:H	O12	1.925	30.39
9f	CF_3	3	ASN81:H VAL79:H VAL79:H	O12 N8 N32	1.919 2.423 2.554	31.76
9g	NO_2	2	VAL79:H ASN81:H	O35 O12	2.532 2.056	33.95

CONCLUSION

In current research work, few analogues of ethyl 3-(5-(2-oxido)-(4-substituted phenoxy)-benzodioxaphosphol-tetrazol-thiophene-2-carboxylates were successfully prepared and characterized. Biological activity and docking studies of these compounds were also conducted. Anti microbial and docking studies reveals that ethyl 3-(5-(2-oxido)-(4-nitro phenoxy)-benzodioxaphosphol-tetrazol-thiophene-2-carboxylate (9g) showing better biological activity. This analogue can be considered as lead compound for further development.

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