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SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL EVALUATION AND DOCKING STUDIES OF 3-(2-(2-OXIDO)-(4-SUBSTITUTED PHENOXY)-BENZO[D]DIOXAPHOSPHOL-4-OXOTHIAZOLIDIN-THIOPHENE-2-CARBOXAMIDES

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ABSTRACT

3-(2-(2-oxido-2-phenoxy/4-substituded phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamides **(9a-g)** were synthesized by condensing 3-(2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide **(7)** with 4-substituted phenyl phosphoro dichloridates**(8a-g)**. The synthon**(7)** was synthesized by hydrolysis of 3-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide **(6)**. The intermediate **(6)** was synthesized by condensing 3-((3,4-dimethoxy benzylidine)amino)thiophene-2-carboxamide**(5)** with mercaptoacetic acid in dioxane. The synthon**(5)** was synthesized by reaction between 3-aminothiophene-2-caroxamide**(3)** and 3,4-dimethoxybenzaldehyde**(4)**. Starting intermediate **(3)** was synthesized by condensation reaction between 2-cyano acetamide**(1)** and 1,4-dithiane-2,5-diol**(2)**. The reagents and conditions were shown in a, b, c, d and e. The synthetic route was shown in **Scheme-I.** The target molecules **(9a-g)** were characterized by IR, ^{1}HNMR, C^{13}NMR, Mass and elemental analysis. The target molecules were subjected to biological evaluation and molecular docking studies. The results observed in the present investigation were reported in this present research article.

KEYWORDS: Mercapto acetic acid, target molecule, Biological evaluation, molecular docking.

INTRODUCTION

Phosphorus Chemistry has pioneered the application of nano[1] techniques and several organo phosphorus compounds have been synthesized to be used as insecticides[2], herbicides[2], fungicides[2], plant growth regulators[3], biological activity against broad spectrum of the bacteria and different kinds of pests and virus[4]. When compared to other chemical class of pesticides, organo phosphorus pesticides were relatively safe and eco-friendly as they were easily degradable in environment after discharging their functions as pesticides. Further, the residue in water and soil act as fertilizers and nutrients.

Variety of heterocyclic products including drugs[5], dyes and intermediates such as thiazol yellow, thioflavin T., thidiazuron[6], herbicides[7], insecticides[8] etc attributed to possess thiazolidin-4-one. Besides the above applications, thiazolidinone moiety is also assoiciated with broad spectrum of biological activities including antibacterial[9], antifungal[10], anti-inflammatory1[11], hypnotic, anticonvulsant, antitubercular[12], antivital[13], antihistaminic[14], anthelmintic, cardiovascular and anticancer[13] Thus different (4-substituted phenoxy)-benzo[d]dioxaphosphol-4-oxothiazolidin-thiophene-2-carboxamides **(9a-g)** were synthesized. The structures of these compounds have been established by IR, ^{1}HNMR, C^{13}NMR, P^{31} and Mass spectral studies. All the new compounds were screened for antimicrobial activity and docking studies.

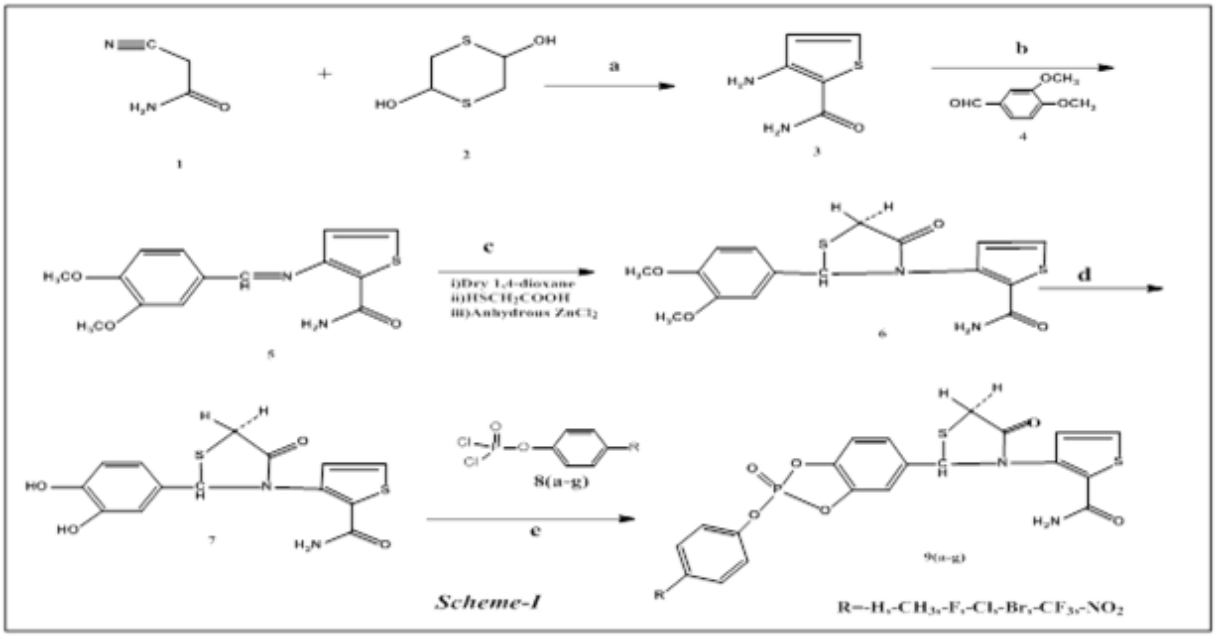
MATERIALS AND METHODS

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. Column chromatography was performed on silica gel with different solvent systems as eluents to afford the pure compound. 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-3000 spectrometer operating at 400MHz for ^{1}H-NMR and 75MHz for ^{13}C-NMR. ^{31}P-NMR spectra were 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.

RESULTS AND DISCUSSION

Proposed synthetic scheme for the preparation of (9a-g) was reported in 5 steps and presented in the **Scheme-I**.



Scheme-I

Compound 9	a	b	c	d	e	f	g
R	-H	-CH_3	-F	-Cl	-Br	-CF_3	-NO_2

Synthesis of 3-aminothiophene-2-carboxamide (3)[15,16]: A solution of 2-cyano acetamide (**1**, 0.02moles), 2,5-dihydroxy-1,4-dithiane(**2**,0.025moles) in ethanol(50ml) was refluxed in the presence of catalytic amount of triethyl amine for 8 hours. After the reaction, the reaction was monitored by TLC using alumina as an adsorbent and 7:3 solvent mixture of n-hexane-ethyl acetate as an eluent. After the completion of reaction, the solvent was evaporated under reduced pressure and the reaction mass kept at room temperature. The isopropyl alcohol was added and maintained the reaction mass at room temperature for 1 hour. The solid was filtered and washed, the wet material with isopropyl alcohol was dried under suction. The residue was recrystallized from 2-propanol. The m.p. of **(3)** was found to be 122-124˚C with a yield of 75%, 0.015moles. The separated solid was identified as 3-aminothiophene-2-carboxamide **(3).** IR (KBr pellet) γ, cm^{-1}: Characteristic bands around 3400 and 3420 str. of $-NH_2$ of amide group, 3345 str. of amine group, 3020 str. of Aromatic proton of thiophene ring, 1670 str. of $-\overset{O}{\overset{\|}{C}}-$ of amide –I band and 1450, 675 characteristic bands of thiophene ring.^{1}H-NMR (δ, ppm): 6.20 s 2H -NH_2 group of amide, 5.20 bs 2H -NH_2 group attached to thiophene ring and 7.1-7.4 m 2H thiophene protons.

Synthesis of 3-((3,4-dimethoxybenzylidene)amino) thiophene-2-carboxamide(5)[17]: Equimolar quantities of 3,4-dimethoxy benzaldehyde (**4**, 0.02moles) and 3-aminothiophene-2-carboxamide(**3**, 0.02moles) were dissolved in absolute alcohol (50ml). To this, three drops of acetic acid was added. The reaction mixture was heated on a steam bath for 5 hours at 100˚C. After the reaction, the reaction was monitored by TLC using Alumina as an adsorbent. The reaction mixture was kept for 24hours at room temperature. The product was dried and recrystallized from warm absolute alcohol. The separated solid was identified as 3-((3,4-dimethoxybenzylidene)amino)thiophene-2-carboxamide**(5).** The m.p. of **(5)** was found to be 160-162◦C with a yield of 75%, 0.015moles. IR(KBr pellet) γ, cm^{-1}**:** Characteristic bands around 3400 and 3420 str. of $-NH_2$ of amide group, 3040 str. of aromatic protons of thiophene ring, 1670 str. of $-\overset{O}{\overset{\|}{C}}-$ of amide –I band, 1620 str. of -CH=N- of azo methine, 1450, 675 characteristic bands of thiophene ring and 1050 $\delta_{-c-o-c-}$ of aromatic ether. ^{1}H-NMR(δ, ppm): 3.80 s 6H two $-OCH_3$ groups, 6.20 s 2H $-NH_2$ of amide group $-\overset{O}{\overset{\|}{C}}-NH_2$ and 7.0-7.40 m 5H C_6H_3 of Benzene ring and 2H of thiophene ring and 8.30 s H C-H of azomethine group).

Synthesis of 3-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide (6)[18,19]: A mixture of 3-((3,4-dimethoxybenzylidine)amino)thiophene-2-carboxamide(**5,** 0.02moles) and mercaptoacetic acid

(0.02moles) was dissolved in dioxane (20ml). To this, Zinc Chloride (0.5mg) was added and refluxed for 8hours. The progress of the reaction was monitored by TLC using cyclohexane and ethylacetate (7:3) solvent mixture as an eluent. The reaction mixture was cooled and resulting solid was washed with sodium bicarbonate solution and recrystallized from absolute alcohol. The separated solid was identified as 3-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide**(6)**. The m.p. ◦C(136-138 ◦C) and yield(75%, 0.015moles). IR(KBr pellet) γ, cm^{-1}**:** 3420 str. $–NH_2$ of amide group, 3040 str. of aromatic protons of thiophene ring, 1765 cm^{-1} str. of >C=O group of thiazolidin-4-one, 1670 str. of —C— of amide –I band, 1450, 675 characteristic bands of thiophene ring, 1415 stretching C-N of thiazolidin-4-one ring, 1050 $\delta_{-c-o-c-}$ of aromatic ether and 690 str. of C-S. ^{1}H-NMR(δ, ppm): 3.80 s 6H two $–OCH_3$ groups, 3.85 d 1H Ha of CH_2 of thiazolidinone ring, 3.97 d 1H H_b of CH_2 of thiazolidinone ring, 5.90 s 1H -CH- of thiazolidinone ring, 6.20 s 2H $-NH_2$ group of amide group , —C—NH₂ and 7.0-7.4 m 5H C_6H_3 of Benzene ring and 2H of thiophene ring.

Synthesis of 3-(2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide(7)[20]:
A solution of 3-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide (**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 ethylacetate, each time 25ml, the organic layer was merged and dried by anhydrous Na_2SO_4. It was then purified by column chromatography (eluent Petroleum ether**:** Ethyl acetate 8:2) to give the product 3-(2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide**(7).** The m.p. of **(7)** was found to be 203-204◦C with a yield of 75%, 0.015moles. IR(KBr pellet) γ, cm^{-1}**:** 3350 cm^{-1}intramolecular hydrogen bonding str. of -OH, 3400 and 3420 str. of amide $–NH_2$, 3040 str. of aromatic protons of Benzene ring and thiophene ring, 1765 cm^{-1} str. of >C=O group of thiazolidin-4-one, 1670 str. of —C— of amide –I band, 1450 & 675 characteristic bands of thiophene ring, 1415 str. of C-N of thiazolidin-4-one ring and 690 str. of C-S of thiazolidinone ring. ^{1}H-NMR(δ, ppm): 3.80 s 6H two $–OCH_3$ groups, 3.85 d 1H Ha of CH_2 of thiazolidinone ring, 3.97 d 1H H_b of CH_2 of thiazolidinone ring, 5.60 s 2H two-OH groups, 5.90 s 1H -CH- of thiazolidinone ring, 6.20 s 2H $-NH_2$ group of amide group , —C—NH₂ and 7.0-7.4 m 5H C_6H_3 of benzene ring and 2H thiophene ring.

Synthesis of 4-substituted phenyl phosphorodichloridates(8a-g)[2,21-23]: 4-substituted phenyl phosphorodichloridates **(8a-g)**were synthesized as reported in the literature.

General procedure for the synthesis of (4-substituted phenoxy)-benzodioxaphosphol-oxothiazolidin-thiophene-2-carboxamides (9a-g)[24]:
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 3-(2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide(**7**, 0.02moles) and triethylamine (0.04moles) in 30ml of dry toluene and 10ml of Tetra Hydro Furon 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. Triethylamine 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. The m.p. of **(9a)** was found to be 85-87◦C with a yield of 60%, 0.012moles. The separated solid was identified as 3-(2-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide**(9a).**

The similar procedure was adopted to synthesize **(9b-g)** by the reaction between 3-(2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide**(7)** with 4-methyl phenyl phosphorodichloridate **(8b)**, 4-fluoro phenyl phosphorodichloridate **(8c)**, 4-chloro phenyl phosphorodichloridate **(8d),** 4-bromo phenyl phosphorodichloridate **(8e)**, 4-(trifluoromethyl) phenyl phosphorodichloridate **(8f)** and 4-nitro phenyl phosphorodichloridate (**8g).**

Spectral, Physical and Analytical data for the compounds (9a-g)
9a: Yield: 60%. m.p:85-87°C. Anal. Found for $C_{20}H_{15}N_2O_6PS_2$ (%): C 50.03, H 2.52, N 5.38, P 6.02 and S 12.92. IR, KBr pellet (γ, cm^{-1}): 3400 & 3450 $-NH_2$ of amide group, 3030 aromatic protons of Benzene ring and thiophene ring, 1765 stretching vibration of —C— group of thiazolidinone, 1670 str. of —C— of amide –I Band, 1415 str. of C-N of thiazolidin-4-one ring, 1250 str. of P=O, 690 str. of C-S of thiazolidinone, 950 str. of P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 3.85 d 1H H_a of CH_2 of thiazolidinone ring, 3.97 d,1H, H_b of CH_2 of thiazolidinone ring, 5.93 s 1H -CH-Ar of thiazolidinone ring, 7.85 s 2H $-NH_2$ group of amide group —C—NH₂ and

6.70 -8.10 m 10H C_6H_3,C_6H_5 and two thiophene protons. ^{13}C-NMR (δ, ppm): 112.2, 134.8, 118.0, 145.0, 171.2, 33.5, 72.9,133.8 ,115.8, 145.1,143.6, 117.2,122.7, 150.2, 120.3, 130.1, 121.3,130.1, 120.3 and 162.3 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}, $C_{15,}$ $C_{16,}$ $C_{17,}$ $C_{18,}$ C_{19} and C_{20} respectively. P^{31}-NMR (δ, ppm): -8.3. Mass (m/z %): 474 $\overline{M}^{\cdot+}$.

9b: Yield: 60%. m.p:74-76˚C. Anal. Found for $C_{21}H_{17}N_2O_6PS_2$ (%): C 50.48, H 2.86, N 5.25, P 5.68 and S 12.50. IR, KBr pellet (γ, cm^{-1}): 3410 & 3430 $-NH_2$ of amide group, 3025 aromatic protons of Benzene ring and thiophene ring, 1758 stretching vibration of —C(=O)— group of thiazolidinone, 1665 str. of —C(=O)— of amide –I Band, 1410 str. of C-N of thiazolidin-4-one ring, 1245 str. of P=O, 687 str. of C-S of thiazolidinone, 945 str. of P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 3.20 s 3H Ar-CH_3, 3.79 d 1H H_a of CH_2 of thiazolidinone ring, 3.87 d 1H H_b of CH_2 of thiazolidinone ring, 5.93 s 1H -CH-Ar of thiazolidinone ring, 7.85 s 2H $-NH_2$ group of amide group —C(=O)—NH_2 and 6.70 -8.10 m 9H C_6H_3,C_6H_4 and two thiophene protons. P^{31}-NMR (δ, ppm): -7.48.

9c: Yield: 60%. m.p: 64-66˚C. Anal. Found for $C_{20}H_{14}FN_2O_6PS_2$ (%): C 48.25, H 2.32, F 3.28, N 5.19, P 5.75 and S 12.40. IR, KBr pellet (γ, cm^{-1}): 3390 & 3410 $-NH_2$ of amide group, 3040 aromatic protons of Benzene ring and thiophene ring, 1760 stretching vibration of —C(=O)— group of thiazolidinone, 1680 str. of —C(=O)— of amide –I Band, 1420 str. of C-N of thiazolidin-4-one ring, 1255 str. of P=O, 695 str. of C-S of thiazolidinone, 960 str. of P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 3.85 d 1H H_a of CH_2 of thiazolidinone ring, 3.97 d 1H H_b of CH_2 of thiazolidinone ring, 5.93 s 1H -CH-Ar of thiazolidinone ring, 7.85 s 2H $-NH_2$ group of amide group —C(=O)—NH_2 and 6.70 -8.10 m 9H C_6H_3,C_6H_4 and two thiophene protons. P^{31}-NMR (δ, ppm): -7.74.

9d: Yield: 65%. m.p: 91-93˚C. Anal. Found for $C_{20}H_{14}ClN_2O_6PS_2$ (%): C 46.54, H 2.20, N 5.96, P 5.45, S 12.00 and Cl 6.40. IR, KBr pellet (γ, cm^{-1}): 3405 & 3420 $-NH_2$ of amide group, 3035 aromatic protons of Benzene ring and thiophene ring, 1755 stretching vibration of —C(=O)— group of thiazolidinone, 1675 str. of —C(=O)— of amide –I Band, 1417 str. of C-N of thiazolidin-4-one ring, 1253 str. of P=O, 695 str. of C-S of thiazolidinone, 955str. of P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 3.85 d 1H H_a of CH_2 of thiazolidinone ring, 3.97 d,1H, H_b of CH_2 of thiazolidinone ring, 5.93 s 1H -CH-Ar of thiazolidinone ring, 7.85 s 2H $-NH_2$ group of amide group —C(=O)—NH_2 and 6.70 -8.10 m 9H C_6H_3,C_6H_4 and two thiophene protons. P^{31}-NMR (δ, ppm): -7.54.

9e: Yield: 68%. m.p: 98-100˚C. Anal. Found for $C_{20}H_{14}ClN_2O_6PS_2$ (%): C 42.82, H 1.98, N 4.48, P 5.04, S 10.88 and Br 13.78. IR, KBr pellet (γ, cm^{-1}): 3400 & 3425 $-NH_2$ of amide group, 3035 aromatic protons of Benzene ring and thiophene ring, 1762 stretching vibration of —C(=O)— group of thiazolidinone, 1675 str. of —C(=O)— of amide –I Band, 1417 str. of C-N of thiazolidin-4-one ring, 1253 str. of P=O, 695 str. of C-S of thiazolidinone, 955str. of P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 3.85 d 1H H_a of CH_2 of thiazolidinone ring, 3.97 d 1H H_b of CH_2 of thiazolidinone ring, 5.93 s 1H -CH-Ar of thiazolidinone ring, 7.85 s 2H $-NH_2$ group of amide group —C(=O)—NH_2 and 6.70 -8.10 m 9H C_6H_3,C_6H_4 and two thiophene protons. P^{31}-NMR (δ, ppm): -6.84.

9f: Yield: 70%. m.p: 90-92˚C. Anal. Found for $C_{21}H_{14}F_3N_2O_6PS_2$ (%): C 45.86, H 1.96, N 4.48, P 5.12, S 11.27 and F 9.88. IR, KBr pellet (γ, cm^{-1}): 3420 & 3445 $-NH_2$ of amide group, 3030 aromatic protons of Benzene ring and thiophene ring, 1750 stretching vibration of —C(=O)— group of thiazolidinone, 1680 str. of —C(=O)— of amide –I Band, 1420 str. of C-N of thiazolidin-4-one ring, 1260 str. of P=O, 695 str. of C-S of thiazolidinone, 965str. of P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 3.85 d 1H H_a of CH_2 of thiazolidinone ring, 3.97 d 1H H_b of CH_2 of thiazolidinone ring, 5.93 s 1H -CH-Ar of thiazolidinone ring, 7.85 s 2H $-NH_2$ group of amide group —C(=O)—NH_2 and 6.70 -8.10 m 9H C_6H_3,C_6H_4 and two thiophene protons. P^{31}-NMR (δ, ppm): -8.80.

9g: Yield: 68%. m.p: 110-112˚C. Anal. Found for $C_{20}H_{14}N_3O_8PS_2$ (%): C 45.64, H 2.09, N 7.53, P 5.38, S 11.74. IR, KBr pellet (γ, cm^{-1}): 3430 & 3450 $-NH_2$ of amide group, 3040 aromatic protons of Benzene ring and thiophene ring, 1770 stretching vibration of —C(=O)— group of thiazolidinone, 1690 str. of —C(=O)— of amide –I Band, 1425 str. of C-N of thiazolidin-4-one ring, 1270 str. of P=O, 690 str. of C-S of thiazolidinone, 970str. of P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 3.85 d 1H H_a of CH_2 of thiazolidinone ring, 3.97 d 1H H_b of CH_2 of thiazolidinone ring, 5.93 s 1H -CH-Ar of thiazolidinone ring, 7.85 s 2H $-NH_2$ group of amide group —C(=O)—NH_2 and 6.70 -8.10 m 9H C_6H_3,C_6H_4 and two thiophene protons. P^{31}-NMR (δ, ppm): -9.10.

Biological activity: The antimicrobial activity[25] of newly synthesized compounds was performed according to disc diffusion method, as recommended by the

National Committee for Clinical Laboratory.[26] The synthesized compounds were used at the concentration of 250µg/ml. DMF as a solvent.

Antibacterial activity: The antibacterial activity[27] of (4-substituted phenoxy)-benzodioxaphosphol-oxothiazolidin-thiophene-2-carboxamides **(9a-g)** were screened against the Staphylococcus aureus (gram positive), Bacillus cereus, Escherichia coli (gram negative) and Pseudomonas aeruginosa organisms. The 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. Most of the compounds showed moderate to good antibacterial activity against both bacteria under present investigation.

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 2106	Escherichia coli NCCS 2065	Pseudomonas aeruginosa NCCS 2200
1	9a	12	7	8	10
2	9b	10	7	8	9
3	9c	16	10	12	13
4	9d	14	9	11	12
5	9e	13	8	9	11
6	9f	17	11	13	15
7	9g	19	14	15	17
Amoxicillin		21	27	24	22

Antifungal activity: Antifungal activity of final compounds (4-substituted phenoxy)-benzodioxaphosphol-oxothiazolidin-thiophene-2-carboxamides **(9a-g)** were screened against Aspergillus niger, Candida albicans[28] The 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. Most of the compounds showed moderate to good antifungal activity against both fungi.

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	11	10
2	9b	09	08
3	9c	16	14
4	9d	14	12
5	9e	13	11
6	9f	18	15
7	9g	20	17
Ketoconazole		22	25

The order of anti-bacterial and anti-fungal activity was found to be
(9g> 9f >9c > 9d > 9e >9a> 9b).

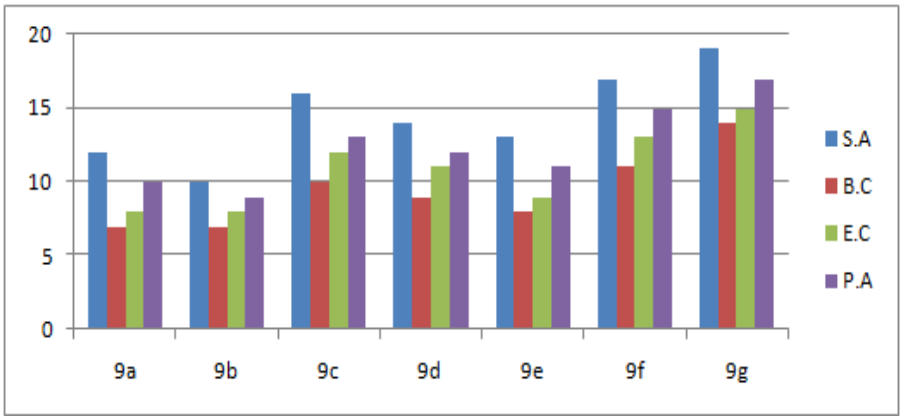


Fig.1. Antibacterial activity of compounds 9(a-g).

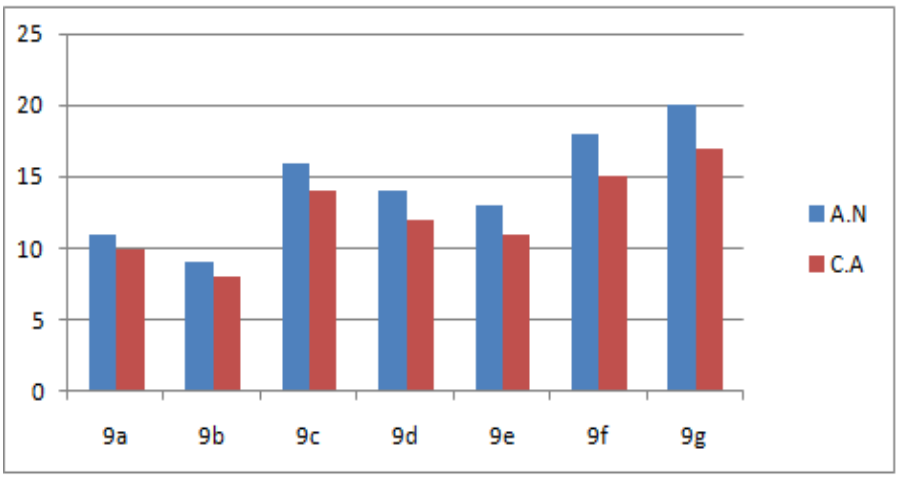
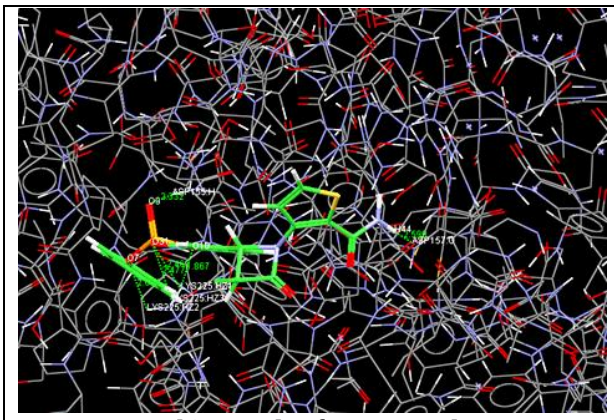


Fig.2: Antifungal activity of compounds 9(a-g).

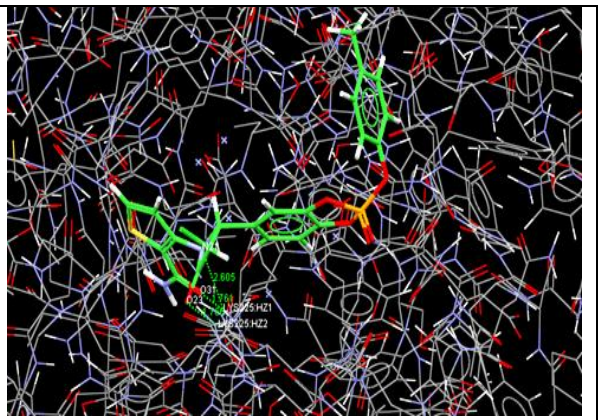
Docking study

Docking[29] of the inhibitors (synthesized compounds from **(9a-g))** with Phytase domain was performed uing GOLD 3.0.1, which is based on Genetic algorithm (GA). The docking studies of **(9a-g)** were carried out on Phytase[30] protein. The docking ligands were found to have some interactions between an oxygen atom of the ligands and Phytase protein. The results pertaining to Docking studies were shown in the **Table-3-Table-4** and in **Fig-3.** Moreover, these docked conformations formed 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 Aspartic acid(157) and Lysine(225). The order of protein-ligand hydrogen bond score is **9b>9f>9e>9g>9a>9d>9c**. Besides hydrogen bonding interaction between ligand-protein, the Van der Waals forces of interactions between ligand-protein were also noticed. The order of protein-ligand Van der Waals score of interaction is found to be **9b>9f>9e>9g>9a>9d>9c** with the protein. However the ligands fail to exhibit intramolecular hydrogen bonding. The ligands exhibit minimum intramolecular strain. Finally, all the ligands exhibit moderate to good antifungal activity with Phytase protein. The order of gold score fitness value of the ligands is found to be **9b>9f>9e>9g>9a>9d>9c**. According to gold score fitness value ligand **9b** exhibits high binding activity with the protein and ligand **9c** shows least binding activity with the protein.

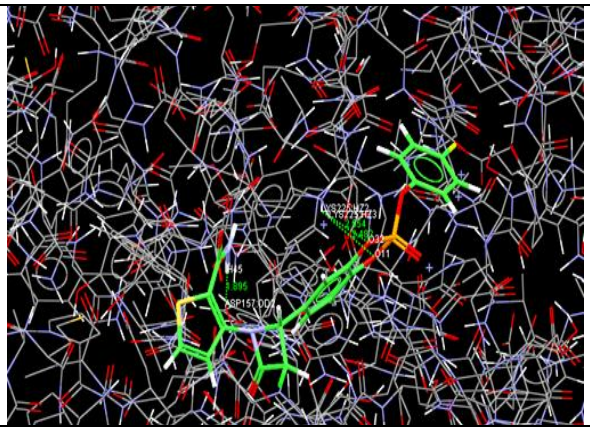
In Gold score evaluation of docking studies, electronic interactions, bonding interactions, steric interaction and conformations of proteins and docked ligand play significant role. However, in the evaluation of antimicrobial studies, electronic factors of the substituents play a significant role.



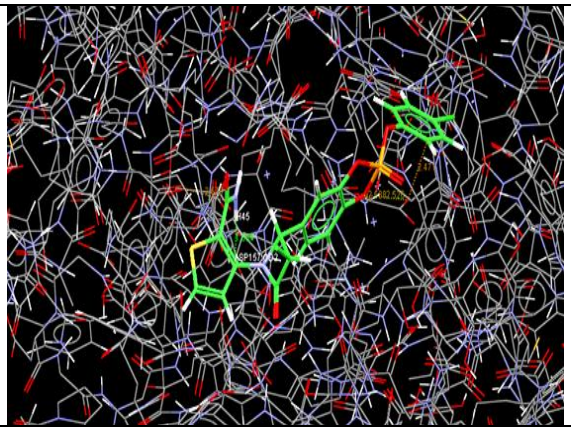
Docking study of Compound 9a



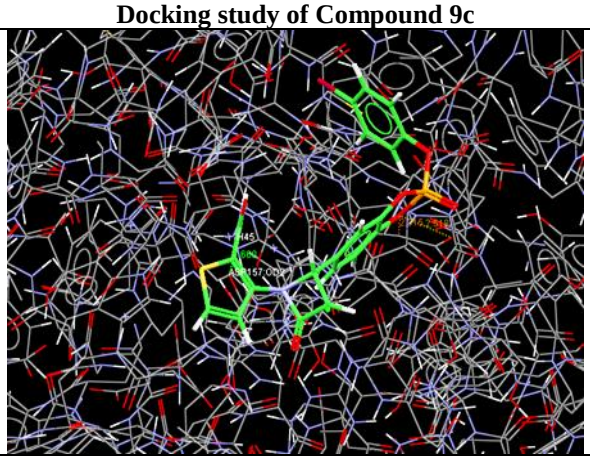
Docking study of Compound 9b



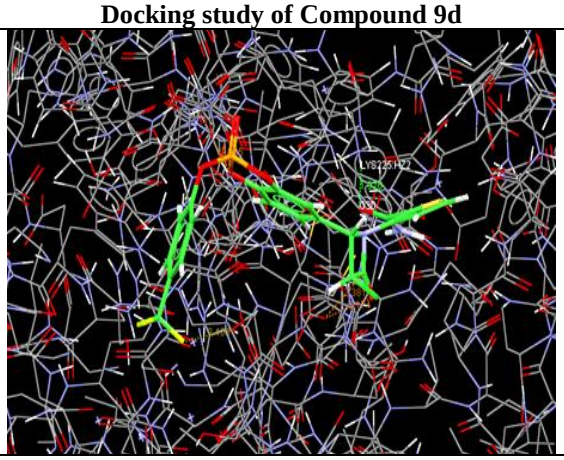
Docking study of Compound 9c



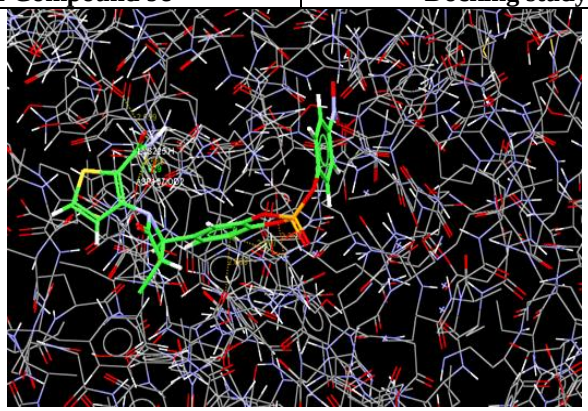
Docking study of Compound 9d



Docking study of Compound 9e



Docking study of Compound 9f



Docking study of Compound 9g

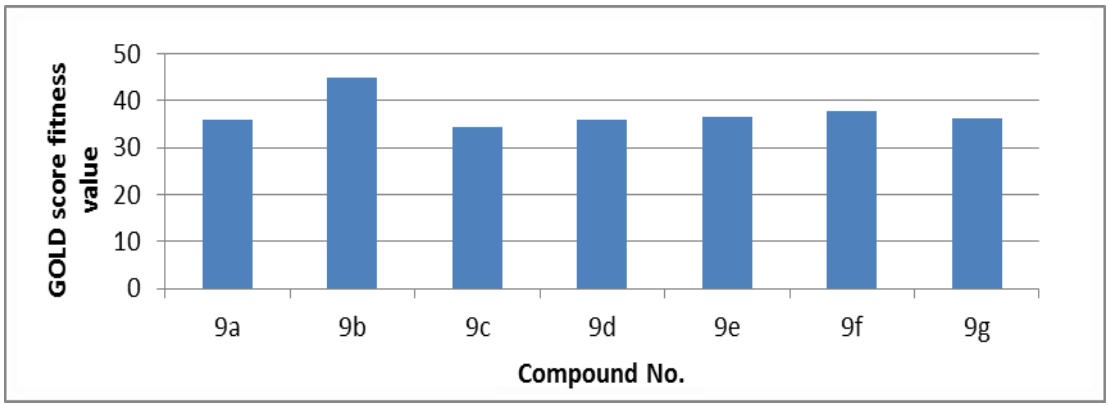


Fig. 3: Comparative Gold Score Fitness values for Compounds (9a-g).

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

Comp	R	Fitness	S(Hb_ext)	S(vdw_ext)	S(Hb_int)	S(vdw_int)
9a	H	36.03	5.82	23.76	0.00	-2.46
9b	CH_3	44.94	6.46	28.78	0.00	-1.09
9c	F	34.25	5.60	24.23	0.00	-4.67
9d	Cl	35.91	5.80	25.20	0.00	-4.55
9e	Br	36.56	6.00	26.42	0.00	-5.77
9f	CF_3	37.92	6.00	24.63	0.00	-1.94
9g	NO_2	36.08	5.86	27.59	0.00	-7.73

Table 4: Hydrogen bonding interactions of Compounds (9a-g) with Phytase protein.

Comp No	R	No of 'H' bonds	Compounds		Bond Length (A^o)	Fitness
			Protein	Atoms		
9a	H	5	ASP157: O LYS225:HZ1 LYS225:HZ2 LYS225:HZ3 LYS225:HZ1	H44 O10 O7 O31 O31	2.599 1.867 2.624 2.460	36.03
9b	CH_3	3	LYS225:HZ1 LYS225:HZ1 LYS225:HZ2	N21 O31 O23	2.605 1.761 1.766	44.94
9c	F	3	LYS225:HZ2 LYS225:HZ3 ASP157: OD2	O11 O32 H45	2.554 2.482 1.895	34.25
9d	Cl	1	ASP157: OD2	H45	3.824	35.90
9e	Br	1	ASP157: OD2	H45	3.660	36.56
9f	CF_3	1	LYS225:HZ2	O30	3.926	37.92
9g	NO_2	1	LYS225: H	O31	1.529	36.07

4. CONCLUSION

In current research work, few anologues of (4-substituted phonoxy)-benzodioxophosphol-oxothiazolidin-thiophene-2-carboxamides were successfully prepared and characterized. Biological activity and docking studies of these compounds were also conducted.

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