ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2021; 7(7): 196-203
www.allresearchjournal.com
Received: 27-04-2021
Accepted: 03-06-2021

YN Spoorthy
Research Scholar, Department of Chemistry, Sri Krishna Devaraya University, Anantapur, Andhra Pradesh, India

P Vijaya Kumar
Research Scholar, Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh, India

T Sailaja Rani
Lecturer, Department of Chemistry, Government College (Men), Anantapur, Andhra Pradesh, India

LK Ravindranath
Professor, Department of Chemistry, Sri Krishna Devaraya University, Anantapur, Andhra Pradesh, India

Corresponding Author:
P Vijaya Kumar
Research Scholar, Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh, India

Synthesis, characterisation, docking studies and anti-microbial evaluation of ethyl (4-substituted phonoxy)-benzodioxophosphol-4-oxothiazolidin-thiophene-2-carboxylates

YN Spoorthy, P Vijaya Kumar, T Sailaja Rani and LK Ravindranath

DOI: https://doi.org/10.22271/allresearch.2021.v7.i7c.8756

Abstract
The reaction of ethyl 3-((3,4-dimethoxybenzylidene)amino) thiophene-2-carboxylate (5) reacts with mercapto acetic acid in presence of zinc chloride in dioxane forms ethyl 3-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide (6) which on hydrolysis affords the ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (7). A new series of ethyl (4-substituted phonoxy)-benzodioxophosphol-4-oxothiazolidin-thiophene-2-carboxylates (9a-g) were synthesized from ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-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, mercapto acetic acid, anti-microbial activity and docking studies

Introduction
Variety of heterocyclic products including drugs [1-2], dyes and intermediates such as thiazol yellow, thioflavin T., thidiazuron [3], herbicides, insecticides [4], activity was attributed to the presence of thiazolidin-4-one moiety. Besides the above applications, thiazolidinones moiety is also associated with broad spectrum of biological activities including anti-bacterial [5-6], anti-fungal [7], anti-inflammatory [8-9], hypnotic, anti-convulsant, anti-tubercular [10], anti-vital [11-13], anti-histaminic [14], anthelmintic, cardiovascular and anti-cancer [15]. In the present studies we have developed a molecular frame, which consists of both organo-phosphorus and thiazolidin-4-one moieties.
Thus different ethyl (4-substituted phonoxy)-benzodioxophosphol-4-oxothiazolidin-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-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (6)

A mixture of ethyl 3-((3,4-dimethoxy benzylidine)amino)thiophene-2-carboxylate (5, 0.02 moles) and mercapto acetic acid (0.02 moles) was dissolved in dioxane (20 ml). To this, Zinc Chloride (0.5 mg) was added and refluxed for 8 hours. The progress of the reaction was monitored by TLC using n-hexane and ethylacetate (7:3) solvent mixture as an elutent. 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 ethyl 3-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxamide (6). The m.p. of (6) was found to be 128-130 °C with a yield of 75%, 0.015 moles.

Yield (75.00%). IR (KBr pellet), ν, cm^{-1}: 3040 cm^{-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), 1765 cm^{-1} (stretching vibration of >C=O group of thiazolidinone), 1695 cm^{-1} (stretching of carbonyl group of an ester), 1450-675 cm^{-1} (characteristics of thiophene ring), 1415 cm^{-1} (stretching of C-N of thiazolidin-2-one ring), 1240 cm^{-1} (stretching of C-O of an ester), 1050 cm^{-1} (δ_{c-o-c} of aromatic ether) and 690 cm^{-1} (stretching vibration of C-S of thiazolidinone ring). ^{1}HNMR (DMSO-d_6), δ, ppm 1.20 (t, 3H, of $-CH_3$ group of ester), 4.20 (q, 2H, $-CH_2$ of an ester), 3.80 (S, 6H, two $-OCH_3$ groups), 3.85 (d, 1H, H_a of CH_2 of thiazolidinone ring), 4.0 (d, 1H, H_b of CH_2 of thiazolidinone ring), 5.93 (s, 1H, -CH-Ar of thiazolidinone ring) and 6.9-7.3(m, 5H, 3H of C_6H_3 ring and two thiophene protons), mp 128-130 °C.

Synthesis of ethyl 3-(2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (7)

A solution of ethyl 3-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (6, 0.02 moles) was dissolved in 30 ml CH_2Cl_2 under N_2 and boron tri bromide (2.4 ml, 0.025 moles) 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 25 ml, 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 ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (7).

Yield (75.00%). IR (KBr pellet), ν, cm^{-1}: 3350 cm^{-1} (intramolecular hydrogen bonding γ_{OH}), 3040 cm^{-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), 1760 cm^{-1} (stretching vibration of >C=O group of thiazolidinone), 1695 cm^{-1} (stretching of carbonyl group of an ester), 1450-675 cm^{-1} (characteristics of thiophene ring), 1415 cm^{-1} (stretching of C-N of thiazolidin-2-one ring), 1240 cm^{-1} (stretching of C-O of an ester) and 690 cm^{-1} (stretching vibration of C-S of thiazolidinone ring). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.2 (t,3H, of $-CH_3$ group of ester), 4.2 (q,2H, $-CH_2$ of an ester), 3.85 (d, 1H, H_a of CH_2 of thiazolidinone ring), 4.0 (d, 1H, H_b of CH_2 of thiazolidinone ring), 5.6 (s, 2H, two –OH group), 5.90 (s, 1H, -CH-Ar of thiazolidinone ring) and 6.9-7.3 (m, 5H, 3H of C_6H_3 ring and two thiophene protons), mp 183-185 °C.

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

4-substituted phenyl phosphoro dichloridates (8a-g) were prepared as perliterature peocedure.

Synthesis of ethyl 3-(2-(2-oxido-2-phenoxybenzo[*d*]/2-(*p*-tolyloxy)benzo[*d*]/2-(4-fluorophenoxy/4-chlorophenoxy/4-bromophenoxy/4-(trifluoromethyl)phenoxy/4-nitrophenoxy)-2-oxidobenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (9a-g) [16]

A solution of phenyl phosphorodichloridate (8a, 0.025 moles) in 25 ml of dry toluene was added drop wise over a period of 20 minutes to a stirred solution of ethyl 3-(2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (7, 0.02 moles) and triethylamine (0.04 moles) in 30 ml of dry toluene and 10 ml 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 2 hours. Later the reaction mixture was heated to 50-60 °C and maintained for 4 hours 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 87-89 °C with a yield of 60%, 0.012 moles. The separated solid was identified as ethyl 3-(2-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxothiazolidin-3-yl) thiophene-2-carboxylate (9a). The structure of the compound (9a) was established by spectral analysis (IR and ^{1}H NMR) and elemental analysis.

The similar procedure was adopted to synthesize (9b-g) by the condensation reaction between ethyl 3-(2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-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}: 3040 cm^{-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), 1760 cm^{-1} (stretching vibration of $-\overset{O}{\overset{\|}{C}}-$ group of thiazolidinone), 1690 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 thiazolidin-2-one ring), 1250 cm^{-1}(stretching vibration of P=O), 1050 cm^{-1} (δ_{c-o-c} of aromatic ether), 950 cm^{-1} (stretching vibration of P-O-$C_{(-Ar)}$ and 690 cm^{-1} (stretching vibration of C-S of thiazolidinone ring). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29 (t, 3H, $-CH_3$ group of an ester), 3.80 (d, 1H, Ha of CH_2 of thiazolidinone ring), 4.0 (d, 1H, H_b of CH_2 of thiazolidinone ring), (q, 2H, $-CH_2$ of an ester), 5.90 (s, 1H, -CH-Ar of thiazolidinone ring), and 7-7.3 (m, 10H, C_6H_3, C_6H_5 and two thiophene protons). ^{13}C-NMR (75MH_Z) (DMSO-d_6), δ, ppm: 101.6, 132.8, 117.6, 141.8, 171.2, 33.5, 73.3, 133.2, 115.8, 145.1, 143.6, 117.2, 122.7, 150.2, 120.3, 130.1, 121.3, 160.5, 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_{14}, C_{15} & C_{19}, C_{16} & C_{18}, C_{17}, C_{20}, C_{21} and C_{22} respectively. ^{31}P-NMR (δ, ppm): -7.60; Mass: 503 (M+1), mp 87-89 °C. Elemental Analysis found for $C_{22}H_{18}NO_7PS_2$ is C: 47.17, H: 2.05, N: 2.30, P: 5.70, S: 12.24.

9b: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3025 cm^{-1} (Ar-H), 2955, 2885 (γ-$_{CH}$ of CH_3 & CH_2), 1685 (C=O), 1758 (C=O, thiazolidine ring), 687 (C-S, thiazolidine 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), 2.20 (s.3H, Ar-CH_3 attached to phenyl ring) 3.80 (d, 1H, Ha of CH_2 of thiazolidinone ring), 4.0 (d, 1H, H_b of CH_2 of thiazolidinone ring), 4.25 (q, 2H, -CH_2 of an ester), 5.90 (s, 1H, -CH-Ar of thiazolidinone ring) and 6.9-7.3 (m, 9H, C_6H_3, C_6H_4 and two thiophene protons); ^{31}P-NMR (δ, ppm): -7.2; Mass: 517 (M+1), mp 92-94 °C. Elemental Analysis found for $C_{23}H_{20}NO_7PS_2$ is C: 52.88, H: 3.35, N: 2.23, P: 5.52, S: 11.90.

9c: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3040 cm^{-1} (Ar-H), 2970, 2900 (γ-$_{CH}$ of CH_3 & CH_2), 1680 (C=O), 1760 (C=O, thiazolidine ring), 695 (C-S, thiazolidine 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), 3.80 (d, 1H, Ha of CH_2 of thiazolidinone ring), 4.0 (d, 1H, H_b of CH_2 of thiazolidinone ring), 4.25 (q,2H, -CH_2 of an ester), 5.90 (s, 1H, -CH-Ar of thiazolidinone ring), and 7.1-7.4 (m, 9H, C_6H_3, C_6H_4 and two thiophene protons); ^{31}P-NMR (δ, ppm): -7.9; Mass: 521 (M+1), mp 75-77 °C. Elemental Analysis found for $C_{22}H_{17}FNO_7PS_2$ is C: 50.18, H: 2.80, F: 3.20, N: 2.26, P: 5.50, S: 11.88.

9d: Yield: 65.00%; IR (KBr pellet), ν, cm^{-1}: 3035 cm^{-1} (Ar-H), 2965, 2895 (γ-$_{CH}$ of CH_3 & CH_2), 1680 (C=O), 1755 (C=O, thiazolidine ring), 691 (C-S, thiazolidine 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), 3.80 (d, 1H, Ha of CH_2 of thiazolidinone ring), 4.0 (d, 1H, H_b of CH_2 of thiazolidinone ring), 4.25 (q, 2H, -CH_2 of an ester), 5.94 (s, 1H, -CH-Ar of thiazolidinone ring), and 7.05-7.3 (m, 9H, C_6H_3, C_6H_4 and two thiophene protons)); ^{31}P-NMR (δ, ppm): -7.2; Mass: 537(M+1), mp 103-105 °C. Elemental Analysis found for $C_{22}H_{17}ClNO_7PS_2$ is C: 48.60, H: 2.76 Cl: 6.16, N: 2.17, P: 5.26, S: 11.40.

9e: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3035 cm^{-1} (Ar-H), 2960, 2890 (γ-$_{CH}$ of CH_3 & CH_2), 1675 (C=O), 1762 (C=O, thiazolidine ring), 697 (C-S, thiazolidine 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), 3.80 (d, 1H, Ha of CH_2 of thiazolidinone ring), 4.0 (d, 1H, H_b of CH_2 of thiazolidinone ring), 4.25 (q, 2H, -CH_2 of an ester), 5.90 (s, 1H, -CH-Ar of thiazolidinone ring), and 7.05-7.3 (m, 9H, C_6H_3, C_6H_4 and two thiophene protons); ^{31}P-NMR (δ, ppm): -6.70; Mass: 583 (M+1), mp 88-90 °C. Elemental Analysis found for $C_{22}H_{17}BrNO_7PS_2$ is C: 48.83, H: 2.51, Br: 13.26, N: 1.96, P: 4.91, S: 10.57.

9f: Yield: 70.00%; IR (KBr pellet), ν, cm^{-1}: 3030 cm^{-1} (Ar-H), 2960, 2900 (γ-$_{CH}$ of CH_3 & CH_2), 1680 (C=O), 1750 (C=O, thiazolidine ring), 695 (C-S, thiazolidine 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), 3.80 (d, 1H, Ha of CH_2 of thiazolidinone ring), 4.0 (d, 1H, H_b of CH_2 of thiazolidinone ring), 4.25 (q, 2H, -CH_2 of an ester), 5.90 (s, 1H, -CH-Ar of thiazolidinone ring), and 7.15-7.4 (m, 9H, C_6H_3, C_6H_4 and two thiophene protons); ^{31}P-NMR (δ, ppm): -8.10; Mass: 571 (M+1), mp 110-112 °C. Elemental Analysis found for $C_{23}H_{17}F_3NO_7PS_2$ is C: 47.83, H: 2.58, F: 9.48 N: 2.02, P: 5.00, S: 10.78.

9g: Yield: 68.00%; IR (KBr pellet), ν, cm^{-1}: 3040 cm^{-1} (Ar-H), 2975, 2905 (γ-$_{CH}$ of CH_3 & CH_2), 1690 (C=O), 1770 (C=O, thiazolidine ring), 690 (C-S, thiazolidine 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), 3.80 (d, 1H, Ha of CH_2 of thiazolidinone ring), 3.90 (d, 1H, H_b of CH_2 of thiazolidinone ring), 4.25 (q, 2H, -CH_2 of an ester), 5.90 (s, 1H, -CH-Ar of thiazolidinone ring), and 7.2-7.40 (m, 9H, C_6H_3, C_6H_4 and two thiophene protons). ^{31}P-NMR (δ, ppm): -8.8; Mass: 548 (M+1), mp 121-123 °C. Elemental Analysis found for $C_{22}H_{17}N_2O_9PS_2$ is C: 47.55, H: 2.68, N: 4.65, P: 5.25, S: 11.26.

Results and Discussion

The synthetic route followed for the synthesis of Ethyl 3-(3-chloro-2-(2-oxido-2-(4-substituted phenoxy)benzo[*d*]-dioxaphosphol-5-yl)-4-oxothiazolidin-3-yl)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 mercapto acetic acid in presence of zinc chloride in dioxane as a solvent affords ethyl 3-(3-chloro-2-(3,4-dimethoxy phenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (6). The IR spectra of ethyl 3-(3-chloro-2-(3,4-dimethoxy phenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (6) exhibited bands around 3040 cm^{-1} (γ-$_{Ar-H}$ of benzene ring and thiophene ring), 1415 cm^{-1} (stretching of C-N of thiazolidin-2-one ring). ^{1}H NMR of (6) showed one singlet at δ 3.80 (s, 6H, two –OCH_3 groups), δ_{ppm}3.85 (d, 1H, H_a of CH_2 of thiazolidinone ring), $\delta_{ppm4.0}$(d, 1H, H_b of CH_2 of thiazolidinone ring), $\delta_{ppm.}$5.93 (s, 1H, -CH-Ar of thiazolidinone ring) 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-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (7) was synthesized by hydrolysis of ethyl 3-(3-chloro-2-(3,4-dimethoxy phenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (6) using boran tri bromide. The IR spectra of Ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (7) exhibited bands around 1760 cm^{-1} (stretching vibration of >C=O group of thiazolidinone), 1415 cm^{-1} (stretching of C-N of thiazolidin-2-one ring). ^{1}H NMR showed one singlet at δ 5.6 (s, 2H, of two –OH groups) confirming the structure of Ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxylate (7).

Ethyl 3-(3-chloro-2-(2-oxido-2-(4-substituted phenoxy)benzo[*d*]-dioxaphosphol-5-yl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylates (9a-g) were prepared by condensing Ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxothiazolidin-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-(3-chloro-2-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (9a) exhibited bands around 1760 cm^{-1} (stretching vibration of —C(=O)— group of thiazolidinone), 1415 cm^{-1} (stretching of C-N of thiazolidin-2-one ring), 1250 cm^{-1} (stretching vibration of P=O), 1050 cm^{-1} ($\delta_{\text{-c-o-c-}}$ of aromatic ether), 950 cm^{-1} (stretching vibration of P-O-$C_{(\text{-Ar})}$ and 690 cm^{-1} (stretching vibration of C-S of thiazolidinone ring). ^{1}H NMR showed multiplet at δ 7.0-7.3(m, 10H, C_6H_3, C_6H_5 and two thiophene protons) confirming the structure of ethyl 3-(3-chloro-2-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxothiazolidin-3-yl)thiophene-2-carboxylate (9a) Similarly remaining analogues (9b-g) were prepared.

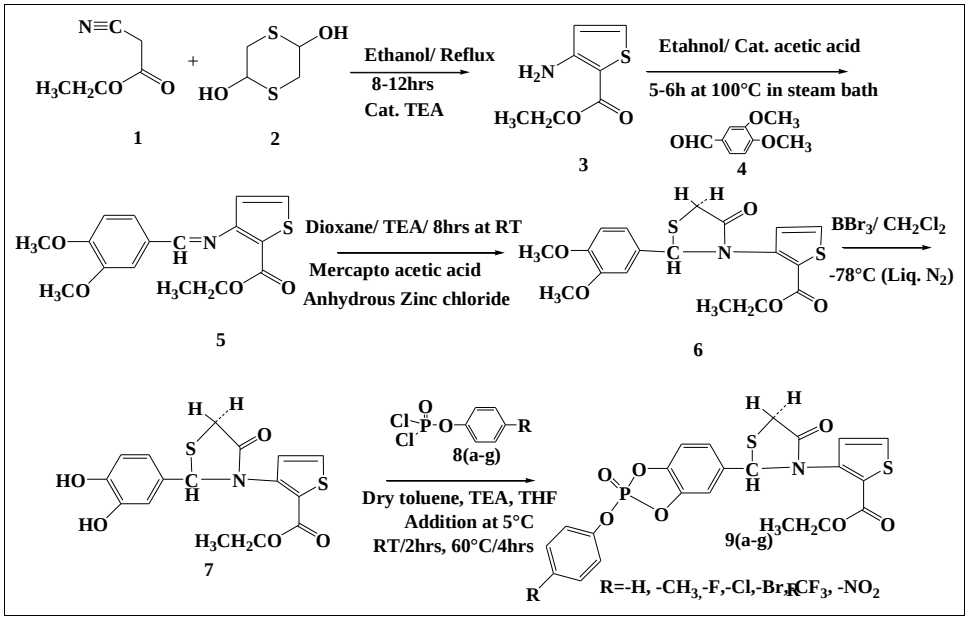


Fig 1: Scheme 1: Synthetic path way for the preparation of (9a-g)

Biological activity

The anti-microbial 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. DMF as a solvent.

Anti-bacterial activity

The anti-bacterial activity of ethyl (4-substitutedphenoxy)-benzodioxaphosphol-4-oxothiazolidin-thiophene-2-carboxylates (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 anti-bacterial 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 anti-bacterial activity against both bacteria under present investigation.

Table 1: Anti-bacterial 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	11	6	7	9
2	9b	9	6	7	8
3	9c	15	9	11	12
4	9d	13	8	10	11
5	9e	12	7	8	10
6	9f	16	10	12	14
7	9g	18	13	14	16
Amoxicillin		21	27	24	22

Anti-fungal activity

Anti-fungal activity of final compounds ethyl (4-substituted phenoxy)-benzodioxaphosphol-4-oxothiazolidin-thiophene-2-carboxylates (9a-g) were screened against *Aspergillus niger*, *Candida albicans*. The substituents nitro (9g), trifluoro methyl (9f) and fluoro (9c) showed more activity than other substituted compounds. The anti-fungal 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 anti-fungal activity against both fungi.

Table 2: Anti-fungal 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	10	9
2	9b	08	07
3	9c	15	13
4	9d	13	11
5	9e	12	10
6	9f	17	14
7	9g	19	16
Ketoconazole		22	25

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

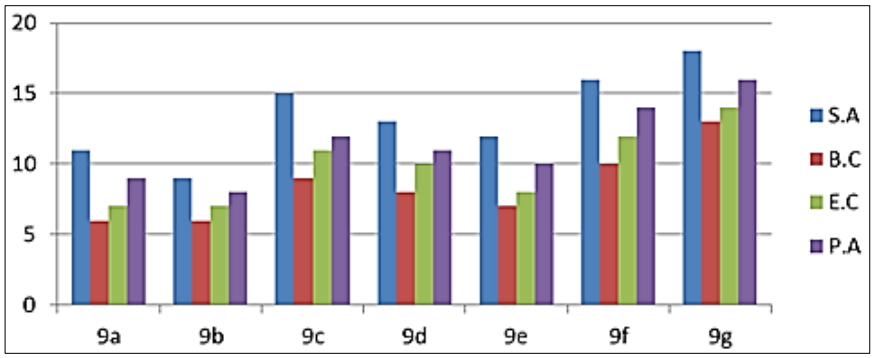


Fig 2: Anti-bacterial activity of compounds (9a-g)

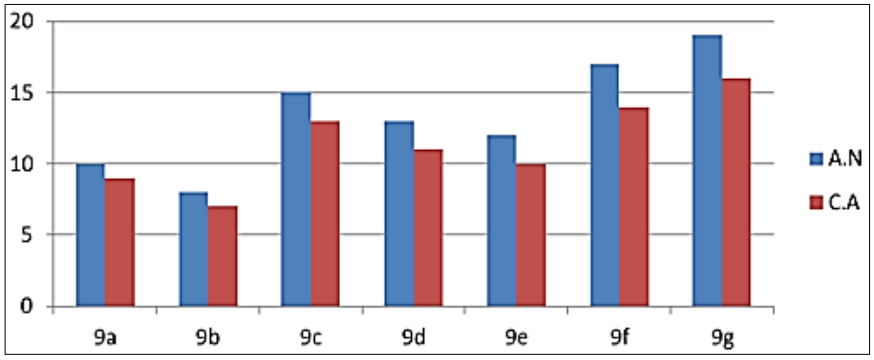
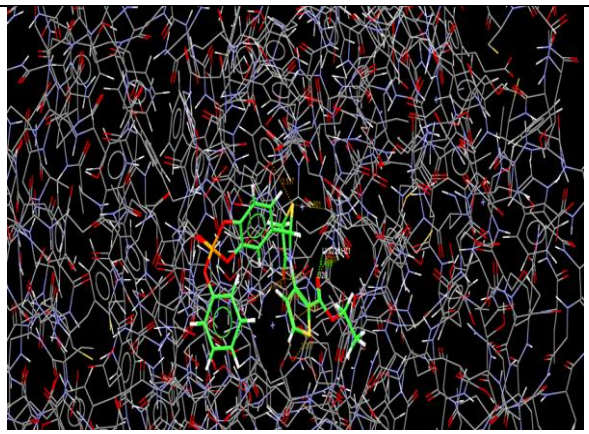


Fig 3: Anti-fungal activity of compounds (9a-g)

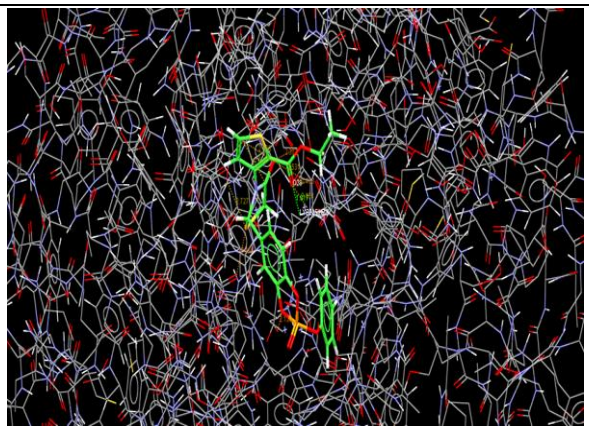
Docking studies of the compounds (9a-g)

Docking of the inhibitors (synthesized compounds from (9a-g)) with Phytase domain was performed using GOLD 3.0.1, which is based on Genetic algorithm (GA). The docking studies of (9a-g) were carried out on Phytase 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 4. 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 Serine (10) and Lysine (225). The order of protein-ligand hydrogen bond score is 9g>9b>9d>9e>9f>9c>9a. 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 9g>9b>9d>9e>9f>9c>9a 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 anti-fungal activity with Phytase protein. The order of gold score fitness value of the ligands is found to be 9g>9b>9d>9e>9f>9c>9a. According to gold score fitness value ligand 9g exhibits high binding activity with the protein and ligand 9a shows least binding activity with the protein.

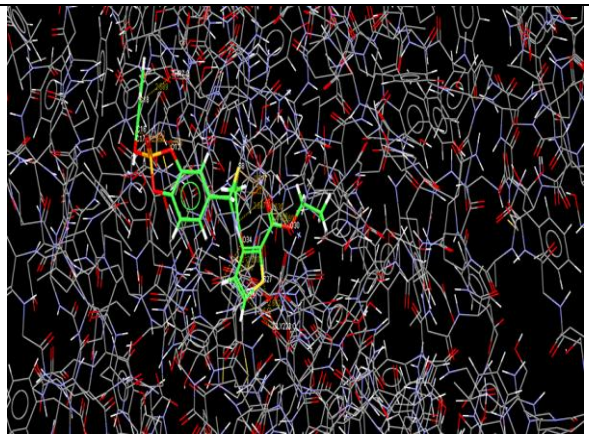
In Gold score evaluation of docking studies, electronic interactions, bonding interactions, steric interaction and conformations of proteins and docked ligand play significant role. However, in the evaluation of anti-microbial studies, electronic factors of the substituents play a significant role.



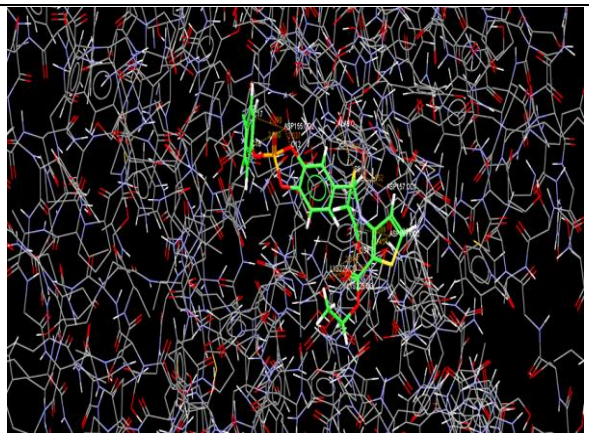
Compound 9a



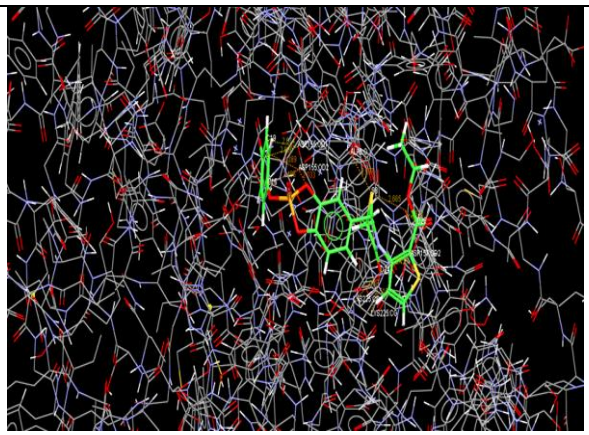
Compound 9b



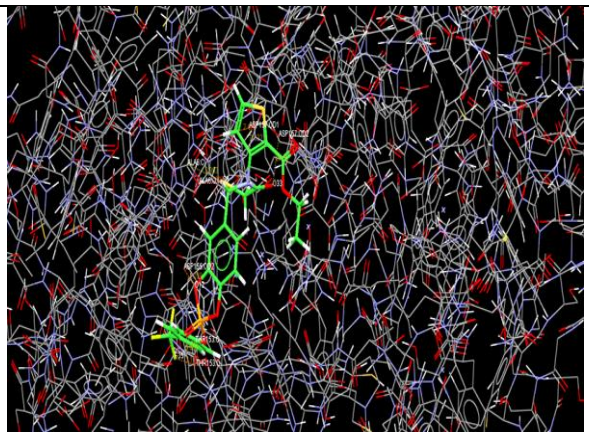
Compound 9c



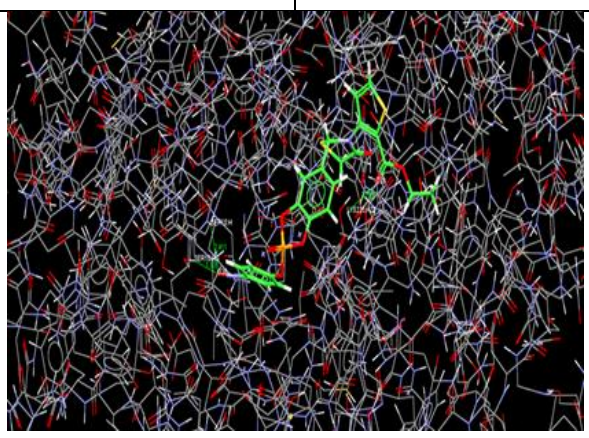
Compound 9d



Compound 9e



Compound 9f



Compound 9g

Fig 4: Docking studies of the compounds (9a-g)

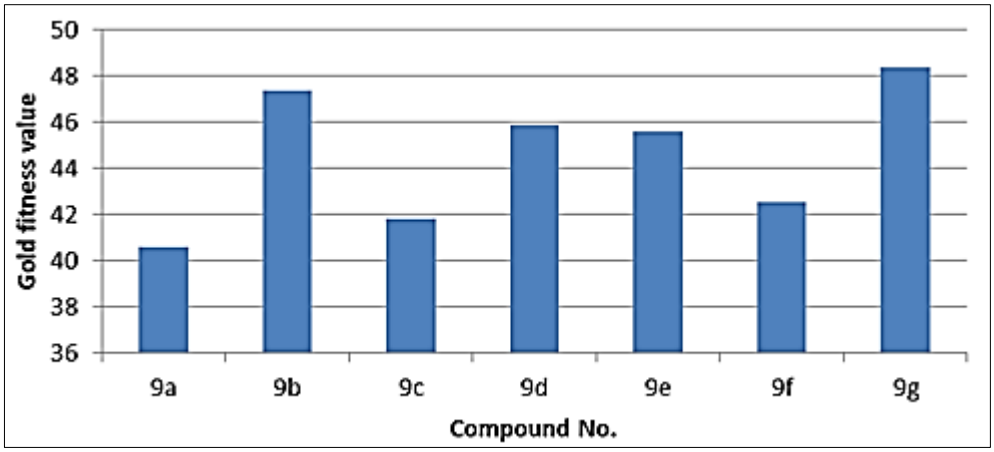


Fig 5: Comparative gold score fitness values for compounds (9a-g)

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

	R	Fitness	S (Hb_ext)	S (vdw_ext)	S (Hb_int)	S (vdw_int)
9a	H	40.59	0.36	29.34	0.00	-0.11
9b	CH_3	47.34	0.00	36.35	0.00	-2.64
9c	F	41.80	0.00	35.74	0.00	-7.35
9d	Cl	45.84	0.00	34.35	0.00	-1.40
9e	Br	45.60	0.00	36.72	0.00	-4.90
9f	CF_3	42.56	0.00	33.12	0.00	-2.98
9g	NO_2	48.39	0.00	35.85	0.00	-0.92

Table 4: Hydrogen bonding interactions of compounds (9a-g) with phytase protein

Comp no.	R	No. of 'H' bonds	Compounds		Bond length (A°)	Fitness
			Protein	Atoms		
9a	H	5	LYS225:HZ1	O28	1.468	40.59
9b	CH_3	1	LYS225:HZ3	O28	1.617	47.34
9c	F		-	-		41.80
9d	Cl		-	-		45.83
9e	Br		-	-		45.60
9f	CF_3		-	-		42.56
9g	NO_2	3	SER10:H SER10:HG LYS225:HZ2	O36 O36 O18	2.411 1.931 1.708	48.37

Conclusion

In current research work, few analogues of ethyl (4-substituted phonoxy)-benzodioxophosphol-4-oxothiazolidin-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 (4-substituted phenoxy)-benzodioxaphosphol-4-oxothiazolidin-thiophene-2-carboxylate (9g) showing better biological activity. This analogue can be considered as lead compound for further development.

Acknowledgements

The authors are thankful to SK University authorities for providing facilities, Anantapur. The authors are also thankful to Central Drug Research Institute, Lucknow, India for supporting to do elemental analysis.

References

1. Nefzi A, Ostresh JM, Houghten RA. The Current Status of Heterocyclic Combinatorial Libraries, Chem. Rev 1997;97:449.
2. Frazen RG. Recent Advances in the Preparation of Heterocycles on Solid Support: A Review of the Literature, J Comb. Chem 2000;2:195.
3. Barreca ML, Clercq ED. Design, Synthesis, Structure-Activity Relationships, and Molecular Modeling Studies of 2,3-Diaryl-1,3-thiazolidin-4-ones as Potent Anti-HIV Agents, J Med. Chem 2002;45:5410.
4. Harris CR, Turnbull SA. Laboratory assessment of the potential of 16 experimental compounds as soil insecticides, Can. Entomol 1977;109:1109.
5. Mohan J, Chadha VK, Chaudhary HS, Sharma BD, Pujari HK, Mohapatra LN. Heterocyclic systems containing bridgehead nitrogen atom. XIII. Anti-fungal and anti-bacterial activities of thiazole and thiazolidinone derivatives, Ind. J Exp. Biol 1972;10:37.
6. Akerblom EB. Synthesis and structure-activity relations of a series of anti-bacterially active 5-(5-nitro-2-furfurylidene)thiazolones, 5-(5-nitro-2-furylpropenylidene)thiazolones, and 6-(5-nitro-2-furyl)-4H-1,3-thiazinones, J Med. Chem 1974;17:609.
7. Dandia A, Singh R, Khaturia S, Merienne C, Morgantc G, Loupyd A. Efficient microwave enhanced region-selective synthesis of a series of benzimidazolyl/triazolyl spiro [indole-thiazolidinones] as potent anti-fungal agents and crystal structure of spiro[3H-indole-3,2'-thiazolidine]-3'(1,2,4-triazol-3-yl)-2,4'(1H)-dione, Bioorg Med. Chem 2006;14:2409.

8. Ottana R, Mazzon E, Dugo L, Monforte F, Maccari R, Sautebin L *et al.* Modeling and biological evaluation of 3,3'-(1,2-ethanediyl)bis[2-(4-methoxyphenyl)-thiazolidin-4-one], a new synthetic cyclooxygenase-2 inhibitor, Eur. J Pharmacol 2002;448:71.
9. Goel B, Kumar A. 2-Substituted-3-(4-bromo-2-carboxyphenyl)-5-methyl-4-thiazolidinones as potential anti-inflammatory agents, Eur. J Med. Chem 1999;34:265.
10. Ottana R, Maccari R, Barreca ML, Bruno G, Rotondo A, Rossi A *et al.* 5-Arylidene-2-imino-4-thiazolidinones: design and synthesis of novel anti-inflammatory agents, Bioorg. Med Chem 2005;13:4243.
11. Karali N, Kocabalkanli A, Gursoy A, Ates O. Synthesis and anti-tubercular activity of 4-(3-coumarinyl)-3-cyclohexyl-4-thiazolin-2-one benzylidenehydrazones, II Farmaco 2002;57:589.
12. Srivastava T, Gaikwad AK, Haq W, Sinha S, Katti SB. Synthesis and biological evaluation of 4-thiazolidinone derivatives as potential anti-mycobacterial agents, ARKIVOC 2005;2:120.
13. Barreca ML. Discovery of 2,3-diaryl-1,3-thiazolidin-4-ones as potent anti-HIV-1 agents, Bioorg. Med. Chem. Lett 2001;11:1793.
14. Diurno MV. Synthesis and structure-activity relationships of 2-(substituted phenyl)-3-[3-(N,N-dimethylamino)propyl]-1,3-thiazolidin-4-ones acting as H1-histamine antagonists, Farmaco 1999;54:579.
15. Gududuru V. Synthesis and anti-proliferative activity of 2-aryl-4-oxo-thiazolidin-3-yl-amides for prostate cancer. Bioorg. Med. Chem. Lett 2004;14:5289.
16. Esther Rani V, Lakshmi Praveena CH, Spoorthi YN, Ravindranath LK. Synthesis, characterization and anti-microbial evaluation of noval compounds 6-nitro-1H-benzo[d]imidazol-2-yl) methyl-6-methoxy-4,8-dihydro-1H-[1,3,2]-dioxaphosphepino[5,6-d]-imidazole-6-oxide –Mannich bases. Der Pharma Chemica 2013;5(3):169-178.