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

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ABSTRACT

The reaction of ethyl 2-cyanoacetate with 2,5-dihydroxy-1,4-dithiane in presence of catalytic amount of tri ethyl amine in ethanol affords ethyl 3-aminothiophene-2- Carboxylate(3). The later on reaction with 3,4-dimethoxy benzaldehyde(4) in presence of few drops of acetic acid to form ethyl 3-((3,4-dimethoxybenzylidene)amino) thiophene-2-Carboxylate(5). Further the inter mediate(5) reacts with Monochloro acetyl chloride in presence of triethyl amine in dioxane forms ethyl 3-(3-chloro-2-(3,4-dimethoxy phenyl)-4-oxoazetidin-1-yl)thiophene-2-Carboxylate(6). Which on hydrolysis affords the ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-Carboxylate(7). A new series of ethyl 3-(3-chloro-2-(2-oxido-2-(4-substituted phenoxy)benzo[*d*]-dioxaphosphol-5-yl)-4-oxoazetidin-1-yl)thiophene-2-carboxylates (9a-g) were synthesized from ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-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, Monochloro acetyl chloride, 4-substituted phenols , antimicrobial activity and docking studies.

INTRODUCTION

Phosphorus Chemistry has pioneered the application of nano and combinatorial techniques in the development of new pharmaceutical material with novel properties. Due to the numerous commercial applications of organo phosphorus compounds, there is an impressive progress in the study of phosphorus chemistry in recent years. Several organo phosphorus compounds have been synthesized to be used as insecticides, herbicides, fungicides, plant growth regulators, biological activity against broad spectrum of the bacteria and different kinds of pests and virus. Organo phosphorus pesticides when compared to other chemical class of pesticides are relatively safe and eco-friendly as they are easily degradable in environment after discharging their functions as pesticides. Further, the residue in water and soil act as fertilizers and nutrients.

Heterocycles possessing azetidin-2-one ring were found to show various types of biological activities.[1-5] Azetidinones are very important class of compounds possessing wide range of biological activities such as antimicrobial,[6-18] pesticidal,[19] antitumor,[20] antitubercular,[21] anticancer,[22] enzyme inhibitors,[23] elastase inhibitors[24] and cholesterol absorption inhibitors.[25] Many β-lactam drugs had been reported in the literature. In the present studies we have developed a molecular frame, which consists of both organo phosphorus and azetidin-2-one moieties. Thus different Ethyl 3-(3-chloro-2-(2-oxido-2-(4-substituted phenoxy)benzo[*d*]-dioxaphosphol-5-yl)-4-oxoazetidin-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-aminothiophene-2-carboxylate (3)

A solution of ethyl 2-cyanoacetate (**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 elutent. 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 separated solid was identified as ethyl 3-aminothiophene-2-carboxylate (3).

Yield (75.00%). IR (KBr pellet), ν, cm^{-1}:3320, 3345, 3040, 2960, 2890, 1695, 1240, 1450, 675. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.20 (3H, t, CH_3), 4.25 (2H, q, CH_2), 6.20 (2H, s, NH_2), 7.1-7.3 (2H, m, Protons of thiophene ring), m.p 112-114°C.

Synthesis of ethyl 3-((3,4-dimethoxybenzylidine)amino)thiophene-2-carboxylate(5)

Equimolar quantities of 3,4-dimethoxy benzaldehyde(4,0.02moles) and ethyl 3-aminothiophene-2-carboxylate (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 5hours 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 ethyl 3-((3,4-dimethoxybenzylidene)amino)thiophene-2-carboxylate(5).

Yield (75.00%). IR (KBr pellet), ν, cm^{-1}:3040, 2960, 2890, 1695, 1620, 1450, 1240, 1050, 675. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.20 (3H, t, CH_3), 4.25 (2H, q, CH_2), 3.80(s,6H,two –OCH_3 groups), 6.90-7.3(m,5H, 3H of C_6H_3 ring and two thiophene protons) and 8.30(s, H, C-H of azo methine group), m.p 150-152 °C.

Synthesis of ethyl 3-(3-chloro-2-(3,4-dimethoxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxylate(6)

Monochloro acetyl chloride (0.025moles) was added drop wise to the compound **(5,** 0.02moles**)** and triethyl amine in dioxane (25ml) at room temperature. The mixture was stirred for 8 hours and left at room temperature for 3-days. Pour the contents on crushed ice to afford ethyl 3-(3-chloro-2-(3,4-dimethoxy phenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxylate **(6).** The product thus formed was filtered and washed with sodium bicarbonate solution. The dried product was recrystallized with absolute alcohol. The separated solid was identified as ethyl 3-(3-chloro-2-(3,4-dimethoxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxylate (6).

Yield (70.00%). 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} (stretching of carbonyl group of an ester), 1670 cm^{-1} (stretching vibration of $>C=O$ group of azetidinone), 1450-675 cm^{-1}(characteristics of thiophene ring), 1415 cm^{-1} (stretching C-N of azetidin-2-one ring), 1240 cm^{-1} (stretching C-O). ^{1}HNMR (DMSO-d_6), δ, ppm 1.2(t,3H, of –CH_3 group of ester), 4.25(q,2H, -CH_2 of an ester), 3.80(S,6H,two –OCH_3 groups), 5.08(d,1H,-CH- of azetidinone ring attached to phenyl ring), 5.44 (d,1H,CH of azetidin attached to -Cl) and 6.9-7.3(m,5H, 3H of C_6H_3 ring and two thiophene protons), mp 138-140°C.

Synthesis of ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)- thiophene-2-carboxylate (7)

A solution of ethyl 3-(3-chloro-2-(3,4-dimethoxyphenyl)-4-oxoazetidin-1-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 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 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} (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), 1670 cm^{-1} (stretching vibration of $>C=O$ group of azetidinone), 1450-675 cm^{-}

[1](characteristics of thiophene ring), 1415 cm^{-1} (stretching of C-N of azetidin-2-one ring), 1240 cm^{-1} (stretching of C-O of an ester). [1]HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.2(t,3H, of $–CH_3$ group of ester),4.25(q,2H, $-CH_2$ of an ester), 5.08(d,1H,-CH- of azetidinone ring attached to phenyl ring),5.6 (s,2H, of two –OH groups), 5.40(d,1H,CH of azetidin attached to –Cl) and 6.9-7.3(m,5H, 3H of C_6H_3 ring and two thiophene protons), mp 127-129°C.

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

4-substituted phenyl phosphoro dichloridates (8a-g) were prepared as perliterature peocedure.[26]

Synthesis of ethyl 3-(3-chloro-2-(2-oxido-2-phenoxy) benzo [*d*]/ 2-(*p*-tolyloxy)benzo[*d*]/-2-(4-fluorophenoxy/4-chlorophenoxy/4-bromophenoxy/4-(trifluoro methyl) phenoxy/ /4-nitro phenoxy) benzo[*d*] [1,3,2] dioxaphosphol-5-yl)-4-oxo azetidin-1-yl)thiophene-2-carboxboxylate (9a-g)

A solution of phenyl phosphorodichloridate (8a, 0.025moles) in 25ml of dry toluene was added drop wise over a period of 20min to a stirred solution of ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-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 4hrs 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 130-132°C with a yield of 60%, 0.012moles. The separated solid was identified as ethyl 3-(3-chloro-2-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxoazetidin-1-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-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxylate (7) with *p*-tolyl 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).

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), 1692 cm^{-1} (stretching vibration of $>C=O$ group of azetidinone), 1670 cm^{-1} (γ —C(=O)— of an ester), 1450-675 cm^{-1}(characteristics of thiophene ring), 1415 cm^{-1}(stretching C-N of azetidin-2-one ring), 830cm^{-1}(δ_{Ar-H} *p*-disubstituted hydrogens of phenyl ring), 740cm^{-1}(o-disubstituted hydrogens of phenyl ring), 672cm^{-1} stretching vibration of **(**C-Cl), 1250cm^{-1} (stretching vibration of P=O) and 950cm^{-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), 4.25 (q,2H, $-CH_2$ of an ester), 5.08(d,1H,-CH- of azetidinone ring attached to phenyl ring), 5.44 (d,1H,CH of azetidinone ring attached to chlorine) and 6.9-7.3(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, 162.2, 62.0, 68.8, 137.5, 114.0, 145.0, 143.2, 117.1, 119.6, 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): -7.0; Mass: 505(M+1), mp 136-138°C. Elemental Analysis found for $C_{22}H_{17}ClNO_7PS$ is C:51.62, H:2.83, Cl:6.52, N:2.30, P:5.68, S:5.85.

9b: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3025cm^{-1} (Ar-H),2955, 2885 (γ_{-CH} of CH_3&CH_2), 1665 (C=O),1410 (C-N), 1245(P=O), 650(C-Cl),945 P-O-C(-Ar); [1]HNMR (DMSO-d_6), δ, ppm (J, Hz):1.29(t,3H, $-CH_3$ group of an ester), 2,34(s,3H,-CH $_3$attached to phenyl ring), 4.25 (q,2H, $-CH_2$ of an ester), 5.08(d,1H,-CH- of azetidinone ring attached to phenyl ring), 5.44 (d,1H,CH of azetidinone ring attached to chlorine), 6.9-7.3(m,9H,C_6H_3,C_6H_4 and two thiophene protons); [31]P-NMR(δ, ppm): -7.7; Mass: 519(M+1), mp 125-127°C. Elemental Analysis found for $C_{23}H_{19}ClNO_7PS$ is C:52.58, H:3.20, Cl:6.32, N:2.28, P:5.39, S:5.67.

9c: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1} (Ar-H),2970, 2900 (γ_{-CH} of CH_3&CH_2), 1680 (C=O),1420 (C-N), 1255(P=O), 670(C-Cl),960 P-O-C(-Ar); [1]HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, $-CH_3$ group of an ester), 4.25 (q,2H, $-CH_2$ of an ester), 5.08(d,1H,-CH- of azetidinone ring attached to phenyl ring), 5.44 (d,1H,CH of azetidinone ring attached to chlorine), 7.0-7.4(m,9H,C_6H_3,C_6H_4 and two thiophene protons); [31]P-NMR(δ, ppm): -7.5; Mass: 513(M+1), mp 113-115°C. Elemental Analysis found for $C_{22}H_{16}ClFNO_7PS$ is C:49.91, H:2.60, Cl:6.31,F:3.11, N:2.18, P:5.55, S:5.68.

9d: Yield: 65.00%; IR (KBr pellet), ν, cm^{-1}: 3035cm^{-1} (Ar-H),2965, 2895 (γ_{-CH} of CH_3&CH_2), 1675 (C=O),1417 (C-N), 1253(P=O), 665(C-Cl),955 P-O-C(-Ar); [1]HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, $-CH_3$ group of an ester), 4.25 (q,2H, $-CH_2$ of an ester), 5.08(d,1H,-CH- of azetidinone ring attached to phenyl ring), 5.44 (d,1H,CH of azetidinone ring attached to chlorine), 7.00-7.73(m,9H,C_6H_3,C_6H_4 and two thiophene protons); [31]P-NMR(δ, ppm): -7.6; Mass: 539(M+1), mp 145-147°C. Elemental Analysis found for

$C_{22}H_{16}Cl_2NO_7PS$ is C:47.32, H:2.53, Cl:12.70, N:2.05, P:5.29, S:5.58.

9e: Yield: 60.00%; IR (KBr pellet), ν, cm^{-1}: 3035cm^{-1} (Ar-H),2960, 2890 (γ_{-CH} of CH_3&CH_2), 1675 (C=O),1417 (C-N), 1253(P=O), 665(C-Cl),955 P-O-C(-Ar); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, -CH_3 group of an ester), 4.25 (q,2H, -CH_2 of an ester), 5.08(d,1H,-CH- of azetidinone ring attached to phenyl ring), 5.44 (d,1H,CH of azetidinone ring attached to chlorine), 7.00-7.30(m,9H,C_6H_3,C_6H_4 and two thiophene protons); ^{31}P-NMR(δ, ppm): -7.5; Mass: 599(M+1), mp 108-110^{o}C. Elemental Analysis found for $C_{22}H_{16}BrClNO_7PS$ is C:44.75, H:2.32, Cl:5.60, Br:1.89 N:1.93, P:4.80, S:5.02.

9f: Yield: 70.00%; IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1} (Ar-H),2970, 2900 (γ_{-CH} of CH_3&CH_2), 1680 (C=O),1420 (C-N), 1260(P=O), 670(C-Cl),965 P-O-C(-Ar); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.29(t,3H, -CH_3 group of an ester), 4.25 (q,2H, -CH_2 of an ester), 5.08(d,1H,-CH- of azetidinone ring attached to phenyl ring), 5.44 (d,1H,CH of azetidinone ring attached to chlorine), 7.2-7.40(m,9H,C_6H_3,C_6H_4 and two thiophene protons); ^{31}P-NMR(δ, ppm): -8.6; Mass: 558(M+1), mp 117-119^{o}C. Elemental Analysis found for $C_{23}H_{16}ClF_3NO_7PS$ is C:47.65, H:2.30, Cl:5.73, F:9.48 N:1.98, P:4.92, S:5.09.

9g: Yield: 68.00%; IR (KBr pellet), ν, cm^{-1}: 3040cm^{-1} (Ar-H),2975, 2905 (γ_{-CH} of CH_3&CH_2), 1690 (C=O),1425 (C-N), 1270(P=O), 675(C-Cl),970 P-O-C(-Ar); ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz):1.29(t,3H, -CH_3 group of an ester),4.25 (q,2H, -CH_2 of an ester), 5.08(d,1H,-CH- of azetidinone ring attached to phenyl ring), 5.44 (d,1H,CH of azetidinone ring attached to chlorine), 7.2-7.40(m,9H,C_6H_3,C_6H_4 and two thiophene protons); ^{31}P-NMR(δ, ppm): -10.2; Mass: 550(M+1), mp 122-124^{o}C. Elemental Analysis found for $C_{22}H_{16}ClN_2O_9PS$ is C:46.42, H:2.45, Cl:6.02, N:4.50, P:5.18, S:5.39.

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-oxoazetidin-1-yl)thiophene-2-carboxylates is presented in scheme-1.

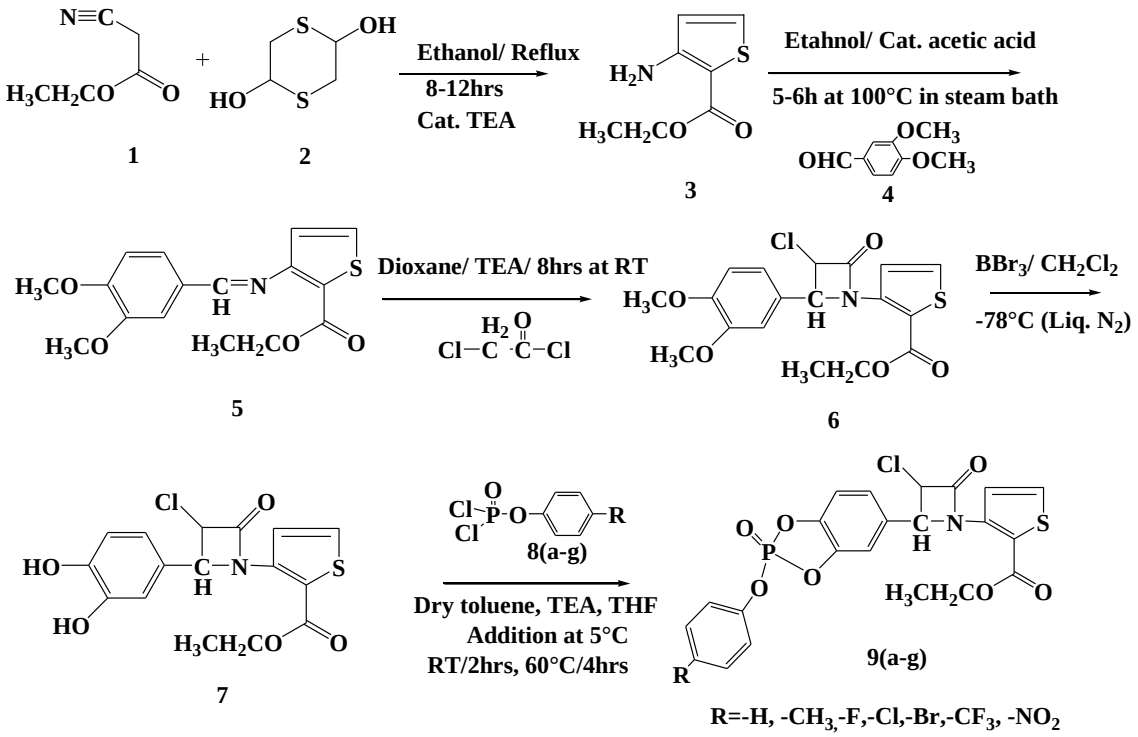
Ethyl 3-aminothiophene-2-carboxylate (3) was prepared by reacting ethyl 2-cyanoacetate with 2,5-dihydroxy-1,4-dithiane in presence of catalytic amount of tri ethyl amine in ethanol at reflux temperature. The IR spectra of ethyl 3-aminothiophene-2-carboxylate (3) exhibited bands around 3320 and 3345 cm^{-1} showing the presence of –NH2 group, bands at 1450 $cm^{-1}$and 675cm^{-1} which is characteristic of thiophene ring. ^{1}H NMR showed one singlet at δ 6.20 (2H, -NH_2), one multiplet at δ 7.1-7.3(m, 2H, Aromatic protons of thiophene ring) confirming the structure of ethyl 3-aminothiophene-2-carboxylate (3).

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 intermediate (5) reacts with mono chloro acetyl chloride in presence of base tri ethyl amine in dioxane as a solvent affords ethyl 3-(3-chloro-2-(3,4-dimethoxy phenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxylate (6). The IR spectra of Ethyl 3-((3,4-dimethoxybenzylidene)amino) thiophene-2-carboxylate(5) exhibited bands around 3040cm^{-1}(γ_{-Ar-H} of benzene ring and thiophene ring), 1620 cm^{-1} ($\gamma_{-CH=N-}$of azo methine). ^{1}H NMR showed one singlet at δ 3.80 (s,6H,two –OCH_3 groups), one multiplet at δ 6.90-7.3(m,5H, 3H of C_6H_3 ring and two thiophene protons) and δ 8.30(s, H, C-H of azo methine group) confirming the structure of Ethyl 3-((3,4-dimethoxybenzylidene)amino) thiophene-2-carboxylate(5). ^{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-oxoazetidin-1-yl)thiophene-2-carbo - -xylate (7) was synthesized by hydrolysis of ethyl 3-(3-chloro-2-(3,4-dimethoxy phenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxylate (6) using boran tri bromide. The IR spectra of Ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxylate (7) exhibited bands around 3350 cm^{-1} (intra molecular hydrogen bonding γ_{-OH}). ^{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-oxoazetidin-1-yl)thiophene-2-carboxylates (9a-g) were prepared by condensing Ethyl 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carbo - -xylate (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-oxoazetidin-1-yl)thiophene-2-carboxylate (9a) exhibited bands around 830cm^{-1}(δ_{Ar-H} *p*-disubstituted hydrogens of phenyl ring),1250cm^{-1} (stretching vibration of P=O) and 950cm^{-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-(3-chloro-2-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxoazetidin-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 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.

Antibacterial activity: The antibacterial activity of (4-substituted phenoxy)-benzodioxaphosphol-oxoazetidin-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.

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	9	4	5	7
2	9b	7	4	5	6
3	9c	13	7	9	10
4	9d	11	6	8	9
5	9e	10	5	6	8
6	9f	14	8	10	12
7	9g	16	11	12	14
Amoxicillin		21	27	24	22

Antifungal activity: Antifungal activity of final compounds (4-substituted phenoxy)-benzodioxaphosphol-oxoazetidin-thiophene-2-carboxamides (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 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	8	7
2	9b	6	5
3	9c	13	11
4	9d	11	9
5	9e	10	8
6	9f	15	12
7	9g	17	14
Ketoconazole		22	25

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

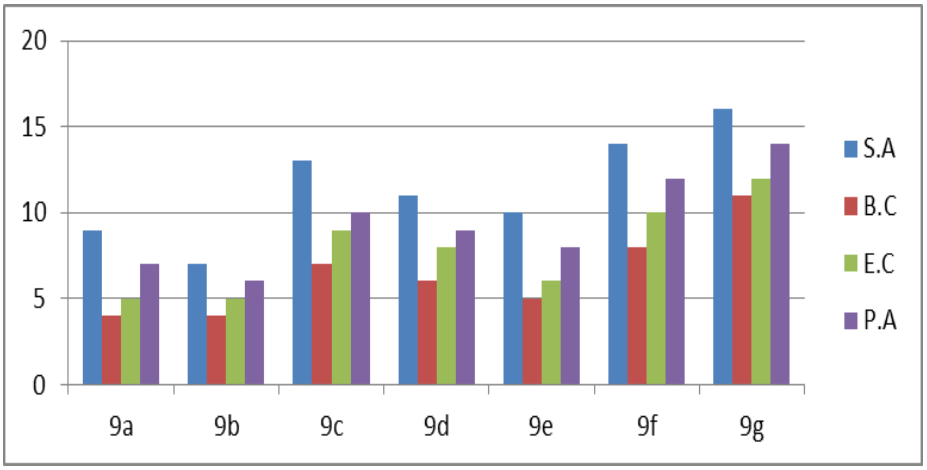


Fig. 1: Antibacterial activity of compounds (9a-g).

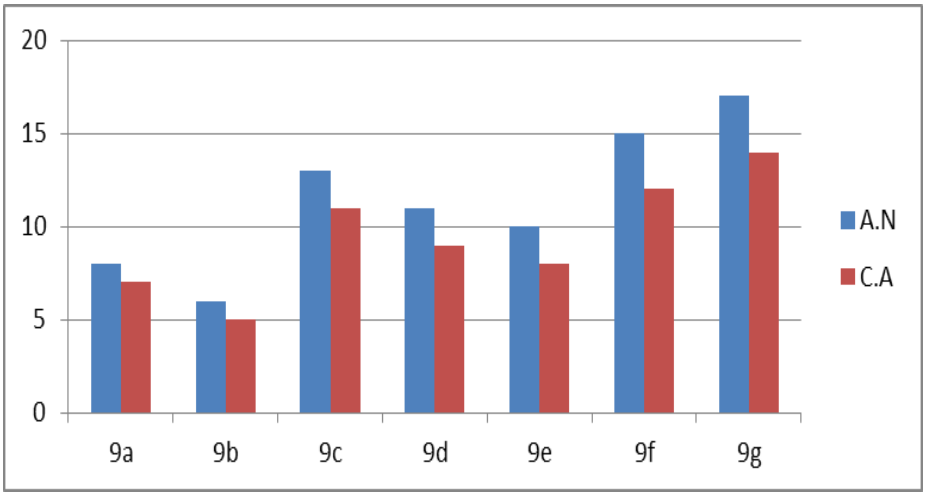


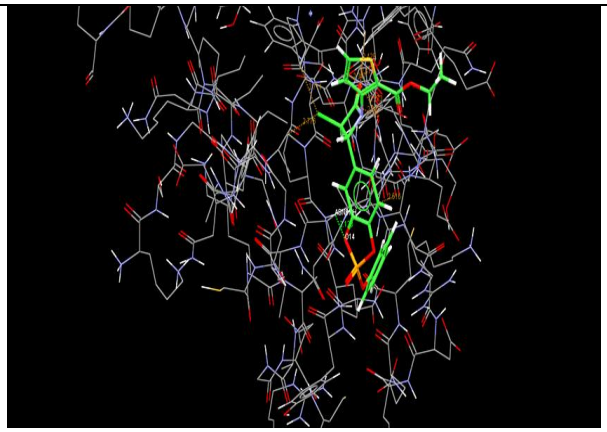
Fig. 2: Antifungal activity of compounds (9a-g).

Docking Studies of the compounds (9a-g)

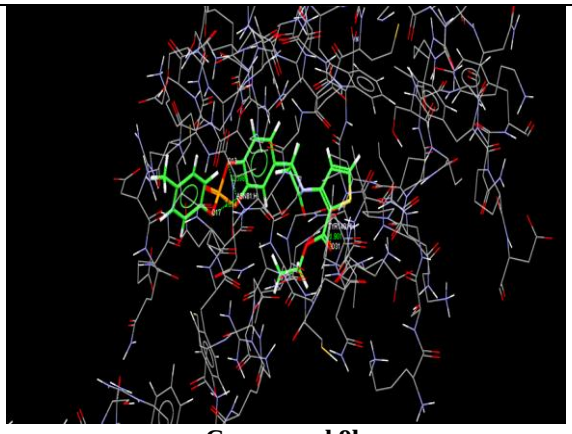
Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). The docking studies of (9a-g) were carried out as model compounds on Peptidoglycan. The docking ligands were found to have some interactions between an oxygen atom of the ligands and Peptidoglycan protein. The results pertaining to Docking studies were shown in the Table-3- Table-4 and in Fig-3. 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), arginine(83), glutamic acid(133), arginine(23) and valine

(79). The order of protein-ligand vanderwaals score of interaction was found to be 9b>9g>9e>9d>9c>9f>9a 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 9b>9g>9e>9d>9c>9f>9a. According to gold score fitness value ligand 9b exhibits high binding activity with the protein and ligand 9a showed least binding activity with the protein. Comparative Gold Score fitness values for (9a-g) were shown in Fig.3.

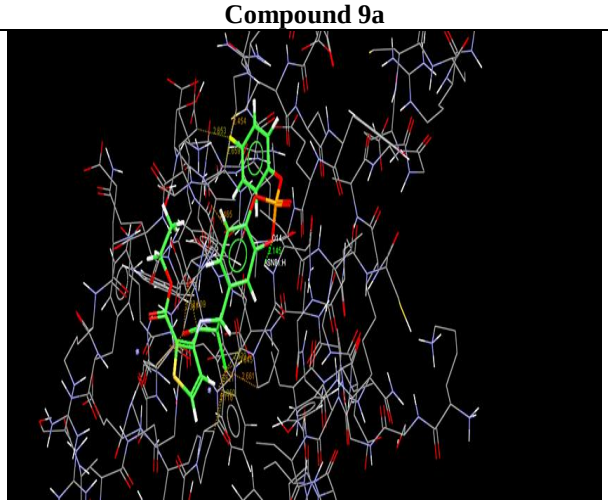
In Gold score evaluation of docking studies, electronic interactions, bonding interactions, steric interaction and conformations of proteins and docked ligand play significant role. However, in the evaluation of antimicrobial studies, electronic factors of the substituents play a significant role.



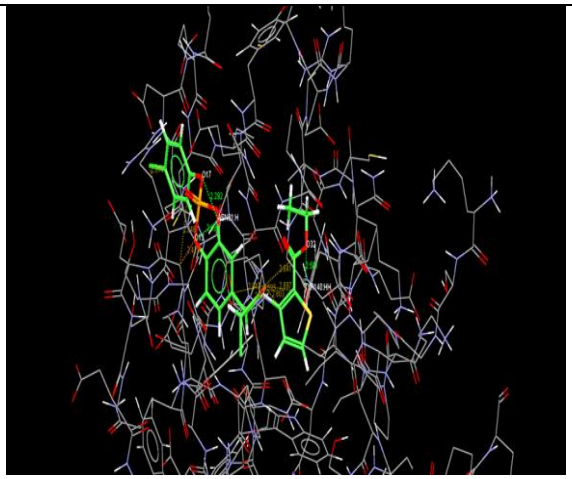
Compound 9a



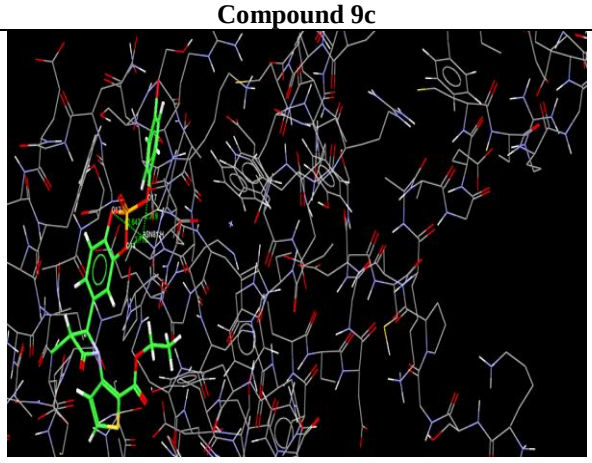
Compound 9b



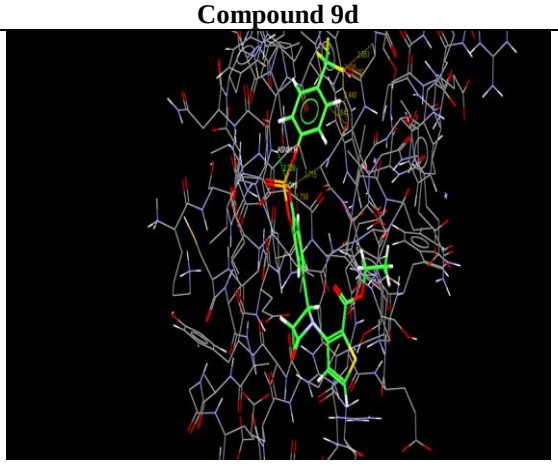
Compound 9c



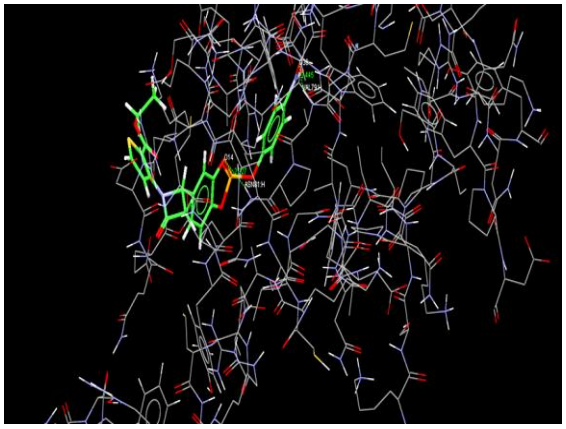
Compound 9d



Compound 9e



Compound 9f



Compound 9g

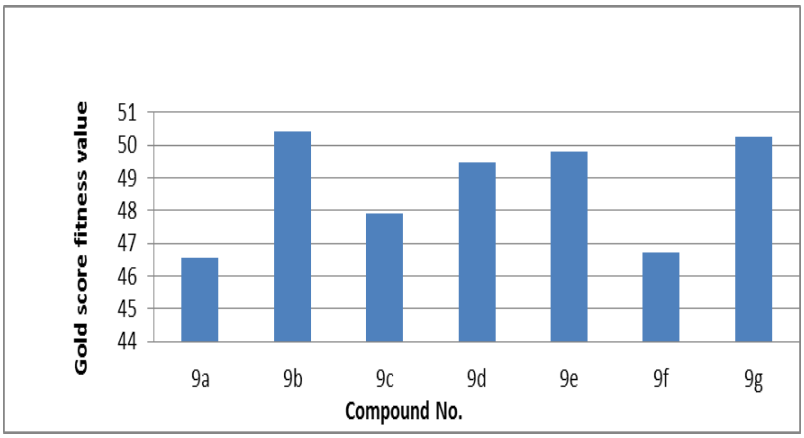


Fig. 3: Comparative gold score fitness values for compounds(9a-g).

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

Comp	R	Fitness	S(Hb_ext)	S(vdw_ext)	S(Hb_int)	S(vdw_int)
9a	H	46.57	0.00	32.99	0.00	1.20
9b	CH_3	50.42	0.00	39.13	0.00	-3.38
9c	F	47.91	0.00	33.41	0.00	1.98
9d	Cl	49.48	0.00	36.68	0.00	-0.96
9e	Br	49.79	0.00	36.07	0.00	0.19
9f	CF_3	46.71	0.00	34.62	0.00	-0.88
9g	NO_2	50.27	0.00	39.28	0.00	-3.73

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

Comp No	R	No of 'H'bonds	Compounds		Bond Length (A^o)	Fitness
			Protein	Atoms		
9a	H	1	ASN81:H	O10	2.123	46.56
9b	CH_3	3	ASN81:H ASN81:H TYR49:H	O13 O17 O31	2.398 2.254 1.901	50.42
9c	F	1	ASN81:H	O14	2.145	47.92
9d	Cl	3	ASN81:H ASN81:H TYR140:H	O17 O13 O32	2.292 2.594 2.561	49.48
9e	Br	3	ASN81:H ASN81:H ASN81:H	O17 O13 O14	2.319 2.847 2.718	49.79
9f	CF_3	1	ASN81:H	O13	2.229	46.72
9g	NO_2	2	VAL79:H ASN81:H	O38 O14	2.445 2.327	50.28

The results of docking study of newly synthesized ethyl (4-substituted phonoxy)-benzodioxophosphol-oxoazetidin-thiophene-2-carboxylates reveals that all the compounds are having good interaction in favourable pose with peptidoglycan protein. Among seven ethyl (4-substituted phonoxy)-benzodioxophosphol-oxoazetidin-thiophene-2-carboxylates, two derivatives (9b&9g) showed better activity.

CONCLUSION

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

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