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Synthesis, Characterization and Antimicrobial Evaluation of 3-(3-chloro)-2-(2-oxido)-(4-substituted phenoxy)-benzo[d]dioxaphosphol-4-oxoazetidin-thiophene-2-carboxamides

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ABSTRACT

3-(3-chloro-2-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxoazetidin-1-yl)thiophene-2-carboxamides (9a-g) were synthesized by condensing 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl) thiophene-2-carboxamide (7) with 4-substituted phenyl phosphoro dichloridates (8a-g). The synthon (7) was synthesized by hydrolysis of 3-(3-chloro-2-(3,4-dimethoxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (6). The intermediate (6) was synthesized by condensing 3-((3,4-dimethoxybenzylidene)amino) thiophene-2-carboxamide (5) with monochloro acetyl chloride. 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 docking studies. The results observed in the present investigation were reported in this present research article.

KEY WORDS: 2-cyanoacetamide, 1,4-dithiane-2,5-diol, 4-substituted phenyl phosphorodichloridates, Monochloro acetyl chloride, condensation reaction, hydrolysis.

1. INTRODUCTION

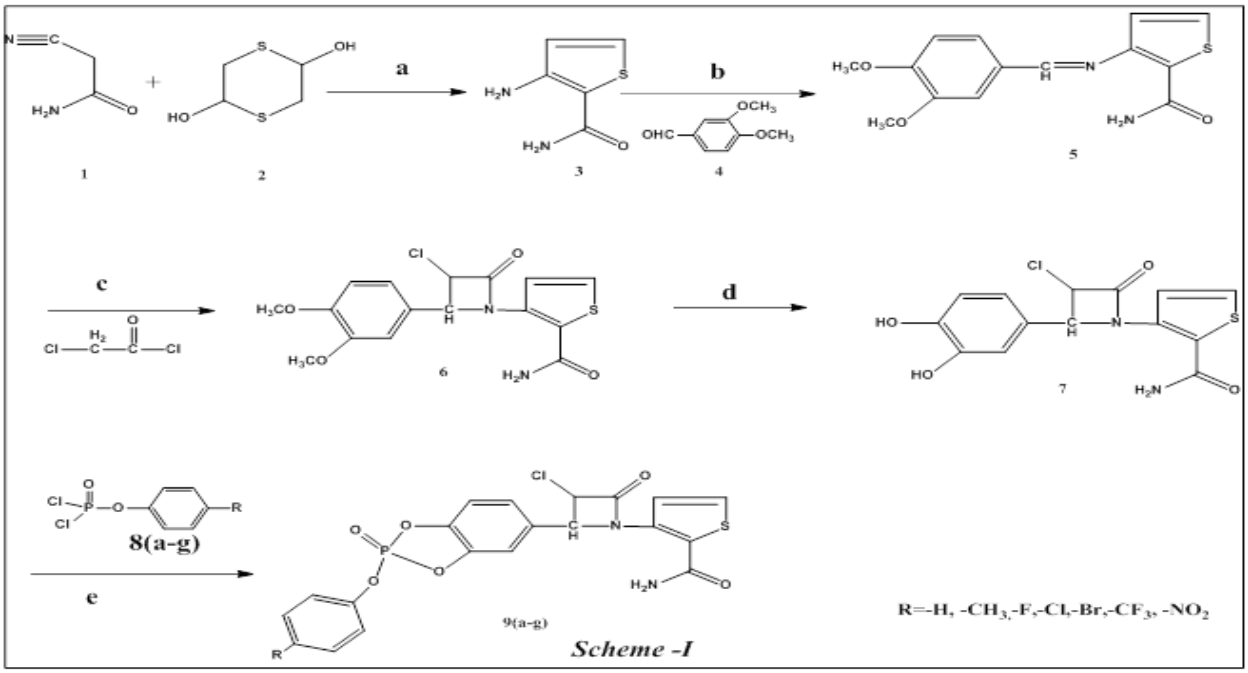
Phosphorus Chemistry has pioneered the application of nano (Vogel, 1978) techniques and several organo phosphorus compounds have been synthesized to be used as insecticides, herbicides, fungicides, plant growth regulators, biological activity against broad spectrum of the bacteria and different kinds of pests and virus (Singh, 1996). 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.

Heterocycles possessing azetidin-2-one ring were found to show various types of biological activities (Desai and Desai, 2005). Azetidinones are very important class of compounds possessing wide range of biological activities such as antimicrobial (Singh, 2010), pesticidal (Singh, 2010), antitumor (Veinberg, 2004), antitubercular (Narute, 2008), anticancer (Banik, 2004), cytotoxic (Maia, 2009), enzyme inhibitors (Gerard S, 2002), elastase inhibitors (Beauve, 2010) and cholesterol absorption inhibitors (Gerard, 2002). Many β-lactam drugs had been reported in the literature.

2. 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-300 spectrometer operating at 400MHz for ^{1}H-NMR and 75MHz for ^{13}C-NMR respectively. ^{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.

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) (Walser, 1991; Hesse, 2007)**:** 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^{0}C with a yield of 75%, 0.015 moles. The separated solid was identified as 3-aminothiophene-2-carboxamide (3).

IR (KBr, 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 carbonyl group 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) (Chhajed, 2010)**:** Equimolar quantities of 3,4-dimethoxy benzaldehyde (4, 0.02 moles) and 3-aminothiophene-2-carboxamide(3, 0.02 moles) 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^{0}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^{0}C with a yield of 75%, 0.015moles.

IR (KBr, cm^{-1}): Characteristic bands around 3400 and 3420 str. of $-NH_2$ of amide group, 3040 str. of aromatic proton of thiophene ring, 1670 str. of carbonyl group 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 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 azo methine group.

Synthesis of 3-(3-chloro-2-(3,4-dimethoxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (6) (Mehta and Shah, 2001; More, 2002)**:** Monochloro acetyl chloride (0.025 moles) was added drop wise to the compound (5, 0.02 moles) 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 3-(3-chloro-2-(3,4-dimethoxy phenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (6). The product thus formed was filtered and washed with sodium bicarbonate solution. The dried product was recrystallized with absolute alcohol. The m.p. of (6) was found to be 150-152^{0}C with a yield of 70%, 0.014 moles. The separated solid was identified as 3-(3-chloro-2-(3,4-dimethoxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (6).

IR (KBr, cm^{-1}): Characteristic bands around 3400 and 3420 str. of-NH_2 of amide group, 3040 str. of protons of thiophene ring, 1690 str. of carbonyl group of azetidinone, 1670 str. of carbonyl group of amide –I band, 1450, 675 characteristic bands of thiophene ring, 1415 stretching C-N of azetidin-2-one ring, 1050 $\delta_{-c-o-c-}$ of aromatic ether and 650 str. of C-Cl.

^{1}H-NMR(δ, ppm): 3.80 s 6H two –OCH_3 groups, 5.10 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone ring, 6.20 s 2H -NH_2 of amide group and 7-7.4 m 5H C_6H_3 and 2H of thiophene ring.

Synthesis of 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (7) (Talon, 2015)**:** A solution of 3-(3-chloro-2-(3,4-dimethoxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (6, 0.02 moles) was dissolved in 30ml CH_2Cl_2 under N_2 and boron tri bromide (2.4ml, 0.025 moles) was added at -78^{0}C (Liq. N_2). 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 (eluent Petroleum ether: Ethyl acetate 8:2) to give the product 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (7). The m.p. of (7) was found to be 150-152^{0}C with a yield of 75%, 0.015 moles.

IR (KBr, cm^{-1}): Characteristic bands around 3350 intra molecular hydrogen bonding str. of -OH, 3400 and 3420 str. of amide –NH_2, 3040 str. of aromatic protons of Benzene ring and thiophene ring, 1690 str. of carbonyl group of azetidinone, 1670 str. of carbonyl group of amide –I Band, 1450 & 650 characteristic bands of thiophene ring, 1415 str. of C-N of azetidin-2-one ring and 670 str. of C-Cl.

^{1}H-NMR(δ, ppm): 5.1 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.60 s 2H two –OH groups, 5.40 d 1H CH of azetidinone ring, 7.5 s 2H -NH_2 group of amide group and 6.9-7.3 m 5H C_6H_3 and 2H of thiophene ring).

Synthesis of 4-substituted phenyl phosphorodichloridates (8a-g) (Jagadeeswara Rao, 2012)**:** 4-substituted phenyl phosphorodichloridates (8a-g) were synthesized as reported in the literature.

General procedure for the synthesis of (4-substituted phenoxy)-benzodioxaphosphol-oxoazetidin-thiophene-2-carboxamides (9a-g) (Esther Rani, 2013)**:** 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 3-(3-chloro-2-(3,4-dihydroxy phenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (7, 0.02 moles**)** and triethyl amine (0.04 moles) in 30ml of dry toluene and 10ml of Tetra Hydro Furan at 5^{o}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^{0}C and maintained for 4hrs with stirring. The completion of the reaction was monitored by TLC analysis. Triethyl amine 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^{0}C with a yield of 60%, 0.012 moles. The separated solid was identified as 3-(3-chloro-2-(2-oxido-2-phenoxybenzo[*d*][1,3,2]dioxaphosphol-5-yl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide (9a).

The similar procedure was adopted to synthesize (9b-g) by the reaction between 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-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).

3. RESULTS AND DISCUSSION

Spectral, Physical and Analytical data for the compounds (9a-g):

9a: Yield: 60%, m.p:130-132^{0}C. Anal. Found for $C_{20}H_{14}ClN_2O_6PS$ (%): C 49.76, H 2.37, Cl 6.92, N 5.25, P 5.78 and S 6.20. IR (γ, cm^{-1}): 3400 & 3450 -NH_2 of amide group, 3040 aromatic protons of Benzene ring and thiophene ring, 1670 carbonyl group of amide –I Band, 1415 C-N of azetidin-2-one ring, 1250 P=O, 660 C-Cl, 950 P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 5.08 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone ring, 7.80 s 2H -NH_2 group of amide group and 6.90 -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, 162.2, 62.0, 68.4, 137.5, 114.0, 144.5, 143.2, 117.1, 119.6, 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): -7.5. Mass (m/z %): 476 M(+1), 478 M(+2).

9b: Yield: 60%, m.p. 145-147^{0}C. Anal. Found for $C_{21}H_{16}ClN_2O_6PS$ (%): C 50.78, H 2.77, Cl 6.63, N 4.91, P 5.73 and S 5.77. IR (γ, cm^{-1}): 3390 & 3410 -NH_2 of amide group, 3025 aromatic protons of Benzene ring and thiophene ring, 1665 carbonyl group of amide –I Band, 1410 C-N of azetidin-2-one ring, 1245 P=O, 650 C-Cl, 945 P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 2,40 s 3H -CH_3 attached to phenyl ring, 5.08 d 1H –CH of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone ring, 7.80 s 2H -NH_2 group of amide group and 6.90-8.10 m 9H C_6H_3 C_6H_4 and two thiophene protons. P^{31}- NMR (δ ppm): -8.2.

9c: Yield: 60%, m.p. 123-125^{0}C. Anal. Found for $C_{20}H_{13}ClFN_2O_6PS$ (%): C 47.95, H 5.60, Cl 6.41, F 3.31, N 5.66, P 5.65 and S 5.98. IR (γ, cm^{-1}): 3410 & 3430 -NH_2 of amide group, 3040 aromatic protons of Benzene ring and thiophene ring, 1680 carbonyl group of amide –I Band, 1420 C-N of azetidin-2-one ring, 1255 P=O, 670 C-Cl, 960 P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 5.08 d,1H, -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone ring, 7.85 s 2H -NH_2 of amide group and 6.90-8.10 m 9H C_6H_3,C_6H_4 and two thiophene protons. P^{31}-NMR (δ ppm): -8.3.

9d: Yield: 65%, m.p. 155-157^{0}C. Anal. Found for $C_{20}H_{13}Cl_2N_2O_6PS$ (%): C 46.32, H 1.96, Cl 13.31, N 4.91, P 5.49 and S 5.68. IR (γ, cm^{-1}): 3405 & 3420 -NH_2 of amide group, 3035 aromatic protons of Benzene ring and thiophene ring, 1675 carbonyl group of amide –I Band, 1417 C-N of azetidin-2-one ring, 1253 P=O, 665 C-Cl, 955 P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 5.08 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone ring, 7.80 s 2H -NH_2 group of amide group and 6.90-8.10 m 9H C_6H_3,C_6H_4 and two thiophene protons. P^{31}-NMR (δ ppm): -8.0.

9e: Yield: 60%, m.p. 118-120^{0}C. Anal. Found for $C_{20}H_{13}BrClN_2O_6PS$ (%): C 42.95, H 1.72, Cl 5.65, N 4.46, P 5.02, S 5.16 and Br 13.68. IR (γ, cm^{-1}): 3400 & 3425 -NH_2 of amide group, 3035 aromatic protons of Benzene ring and thiophene ring, 1675 carbonyl group of amide –I Band, 1417 C-N of azetidin-2-one ring, 1253 P=O, 665 C-Cl, 955 P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 5.08 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone ring, 7.80 s 2H -NH_2 group of amide group and 6.90-8.10 m 9H C_6H_3, C_6H_4 and two thiophene protons. P^{31}-NMR (δ ppm): -7.5.

9f: Yield: 70%, m.p. 109-111^{0}C. Anal. Found for $C_{21}H_{13}ClF_3N_2O_6PS$ (%): C 45.70, H 1.85, Cl 5.83, F 9.87, N 4.62, P 5.09 and S 5.27. IR (γ, cm^{-1}): 3420 & 3447 -NH_2 of amide group, 3030 aromatic protons of Benzene ring and thiophene ring, 1680 carbonyl group of amide –I Band, 1420 C-N of azetidin-2-one ring, 1260 P=O, 670 C-Cl, 965 P-O-$C_{(-Ar)}$. ^{1}H-NMR(δ, ppm): 5.08 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone ring, 7.80 s 2H -NH_2 group of amide group and 6.90-8.10m 9H C_6H_3,C_6H_4 and two thiophene protons. P^{31}-NMR (δ ppm): -9.1.

9g: Yield: 68%, m.p. 137-139^{0}C. Anal. Found for $C_{20}H_{13}ClN_3O_8PS$ (%): C 45.40, H 1.95, Cl 6.25, N 7.43, P 5.34 and S 5.50. IR (γ, cm^{-1}): 3430 & 3450 -NH_2 of amide group, 3040 aromatic protons of Benzene ring and thiophene ring, 1685 carbonyl group of amide –I Band, 1425 C-N of azetidin-2-one ring, 1270 P=O, 675 C-Cl, 970 P-O-$C_{(-Ar)}$. ^{1}H-NMR (δ, ppm): 5.08 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone ring, 7.80 s 2H -NH_2 group of amide group and 6.90-8.10 m 9H C_6H_3, C_6H_4 and two thiophene protons. P^{31}-NMR (δ ppm): -10.9.

Biological activity: The antimicrobial activity (Bhaktavatchala Reddy, 2010) of newly synthesized compounds was performed according to disc diffusion method, as recommended by the National Committee for Clinical Laboratory (Nagalakshmi, 2008). The synthesized compounds were used at the concentration of 250µg/ml. DMF as a solvent.

Antibacterial activity: The antibacterial activity (Balakrishna, 2009) of (4-substituted phenoxy)-benzodioxaphosphol-oxoazetidin-thiophene-2-carboxamides **(**9a-g) were screened against the *Staphylococcus aureu*s (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**	**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**

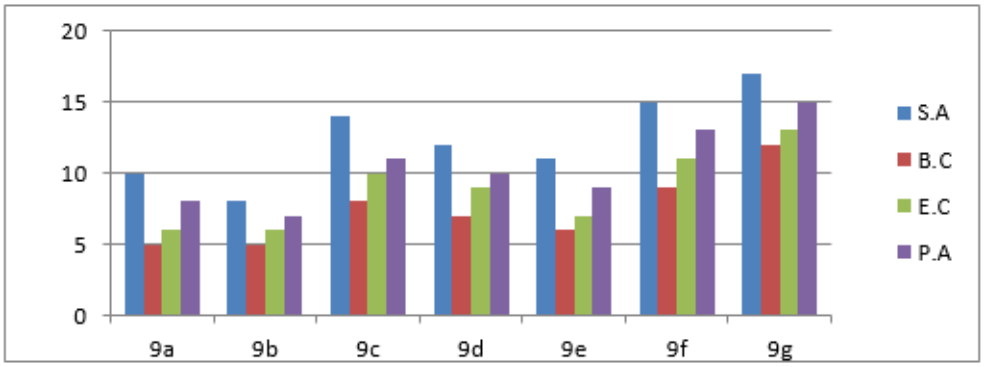


Figure.1. Antibacterial activity of Compounds (9a-g)

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)	
		Aspergillus 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).

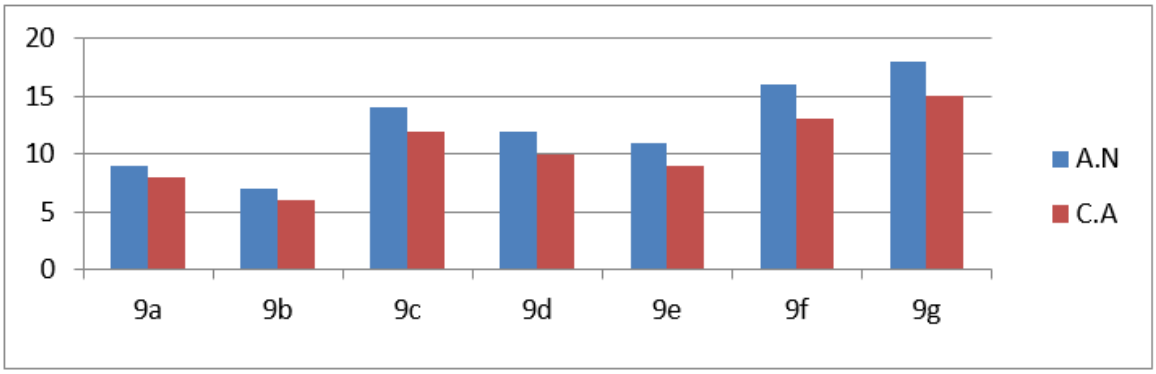
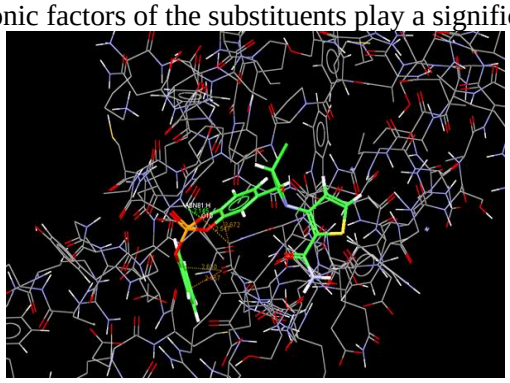


Figure.2. Antifungal activity of Compounds (9a-g)

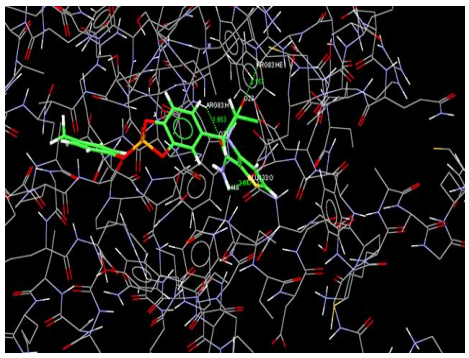
Docking Studies of the compounds (9a-g): Docking (Kurjogi, 2018) was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). The docking studies of (9a-g) were carried out as model compounds on Peptidoglycan (Mohammad Azam Ansari and Sarah Mouse Maadi Asiri, 2021). 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.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), arginine (83), glutamic acid (133), arginine (23) and valine (79). The order of protein-ligand Van der Waals score of interaction was found to be 9g>9b>9f>9c>9e>9d>9a with the protein. However the ligands fail to exhibit intra molecular hydrogen bonding with the ligand. The ligands exhibit minimum intra molecular 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>9b>9f>9c>9e>9d>9a. According to gold score fitness value ligand 9g 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.4.

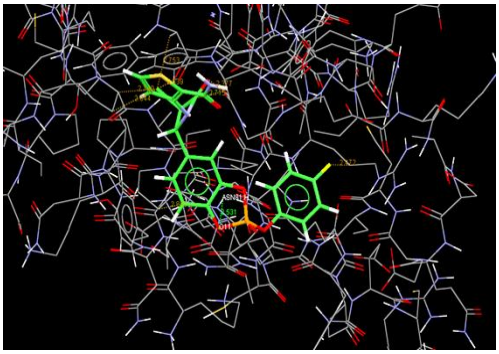
In Gold score evaluation of docking studies, electronic interactions, bonding interactions and steric interaction of the substituents play an important role. However, in the evaluation of antimicrobial studies, electronic factors of the substituents play a significant role.



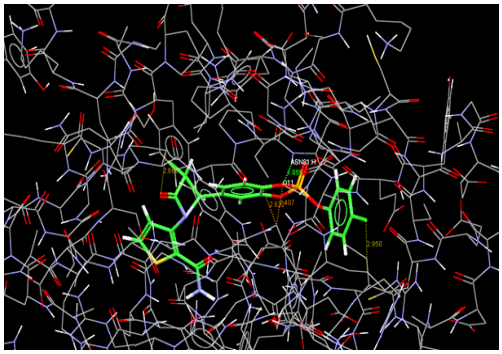
9a



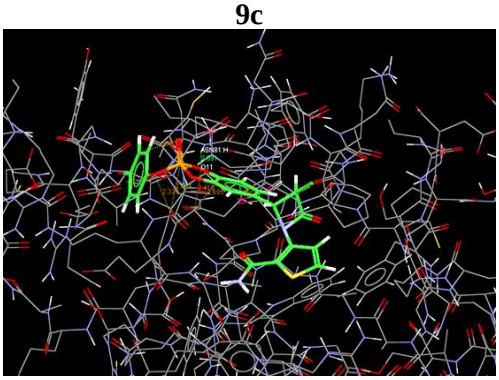
9b



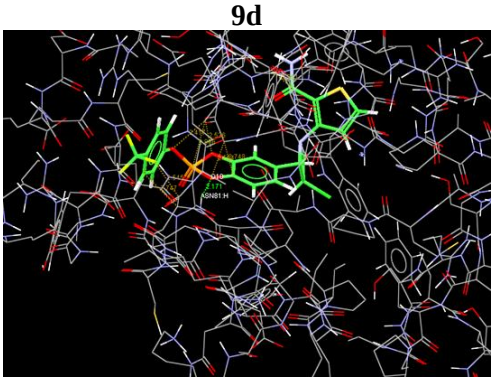
9c



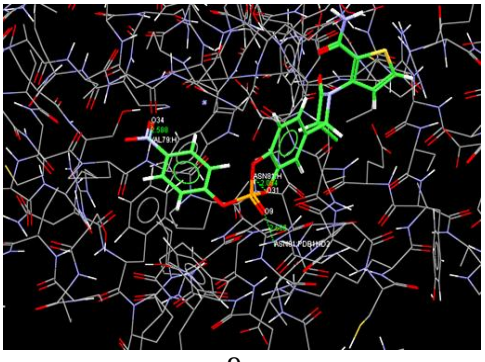
9d



9e



9f



9g

Figure.3. Docking studies of Compounds (9a-g)

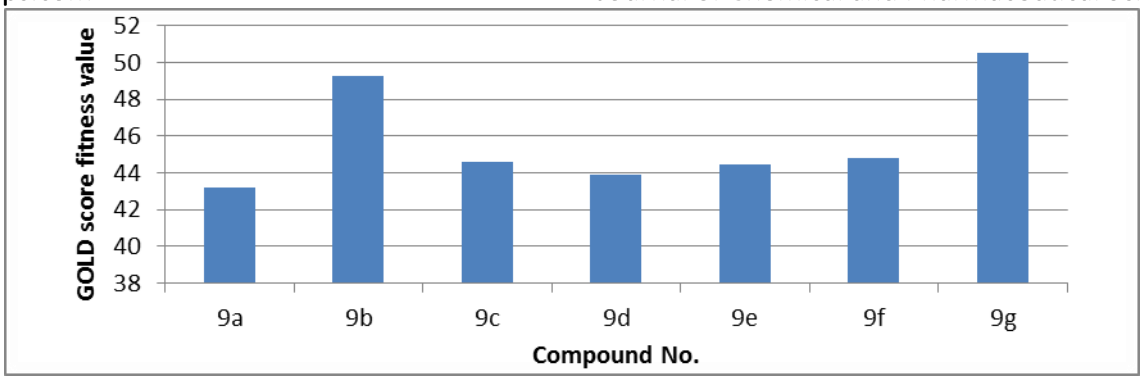


Fig.4. Comparative Gold Score Fitness values for Compounds (9a-g)

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

Comp	R	Fitness	S (Hb_ext)	S (vdw_ext)	S (Hb_int)	S (vdw_int)
9a	H	43.17	0.00	31.88	0.00	-0.66
9b	CH_3	49.28	0.00	38.36	0.00	-3.47
9c	F	44.56	0.00	34.41	0.00	-2.75
9d	Cl	43.87	0.00	33.99	0.00	-2.87
9e	Br	44.46	0.00	34.76	0.00	-3.33
9f	CF_3	44.79	0.00	32.61	0.00	-0.04
9g	NO_2	50.51	0.00	36.25	0.00	0.66

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

Comp No	R	No of 'H' bonds	Compounds		Bond Length (Aº)	Fitness
			Protein	Atoms		
9a	H	1	ASN81:H	O10	2.145	43.17
9b	CH_3	3	ARG83:HE ARG23:H GLU133:O	O23 O31 H48	2.157 1.953 2.047	49.28
9c	F	1	ASN81:H	O11	2.531	44.56
9d	Cl	1	ASN81:H	O11	1.859	43.87
9e	Br	1	ASN81:H	O11	2.091	44.46
9f	CF_3	1	ASN81:H	O10	2.171	44.79
9g	NO_2	3	ASN81:PDBHND2 ASN81:H VAL79:H	O9 O31 O34	2.686 2.094 2.588	50.50

4. CONCLUSION

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

5. ACKNOWLEDGEMENT

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