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SYNTHESIS, CHARACTERIZATION, DOCKING STUDIES AND ANTIMICROBIAL EVALUATION OF 3-(3-CHLORO)-2-(2-OXIDO)-(4-SUBSTITUTED PHENYL)-UREIDO-BENZO[*D*][1,3,2]DIOXAPHOSPHOL-5-YL-4-OXOAZETIDIN-1-YL)THIOPHENE-2-CARBOXAMIDES AND CARBAMOYL THIOPHENE-CHLORO-4-OXOAZETIDIN-DIOXAPHOSPHOL-(MORPHOLINE/PIPERIDINE/4-METHYL PIPERIZINE)-CARBOXAMIDES

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ABSTRACT

3-(3-chloro)-2-(2-oxido)-(4-substituted phenyl)-ureido-benzo[*d*][1,3,2]dioxaphosphol-5-yl-4-oxoazetidin-1-yl)thiophene-2-carboxamides **(9a-f)** were synthesized by condensing 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide**(7)** with 4-substituted phenyl-carbamido-phosphoric acid dichlorides **(8a-f)**. Similarly carbamoyl thiophene-chloro-4-oxoazetidin-dioxaphosphol Morpholine/Piperidine/4-methyl piperizine-carboxamides **(9g-i)** were synthesized by condensing **(7)** with Morpholinyl/Piperidinyl/N-methyl piperizinyl-carbamido-phosphoric acid dichlorides **(8g-i)**. 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 in the presence of triethyl amine in dioxane. The synthon**(5)** was synthesized by reaction between 3-aminothiophene-2-caroxamide**(3)** and 3,4-dimethoxybenzaldehyde**(4)** in the presence of few drops of acetic acid. Starting intermediate **(3)** was synthesized by condensation reaction between 2-cyano acetamide**(1)** and 1,4-dithiane-2,5-diol**(2)** in the presence of catalytic amount of tri ethyl amine in ethanol. The target molecules **(9a-i)** were characterized by IR, ^{1}H-NMR, ^{13}C-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.

KEYWORDS: 2-cyanoacetamide, 1,4-dithiane-2,5-diol, 4-substituted phenyl-carbamido-phosphoric acid dichlorides, Morpholine/Piperidine/4-methyl piperizine-carboxamides, Monochloro acetyl chloride, condensation reaction, hydrolysis.

INTRODUCTION

Organo phosphorus heterocyclic urea derivatives are widely used as anti-infective agents[1], anti-tumor agents, anti-cancer agents and antibacterial agents. Carboxamide derivatives with hetero cyclic moiety proved to inhibit mammalian 5-lipoxygenase enzyme and their usefulness in the treatment of asthma, allergic disorders and cardiovascular disorder.[2]

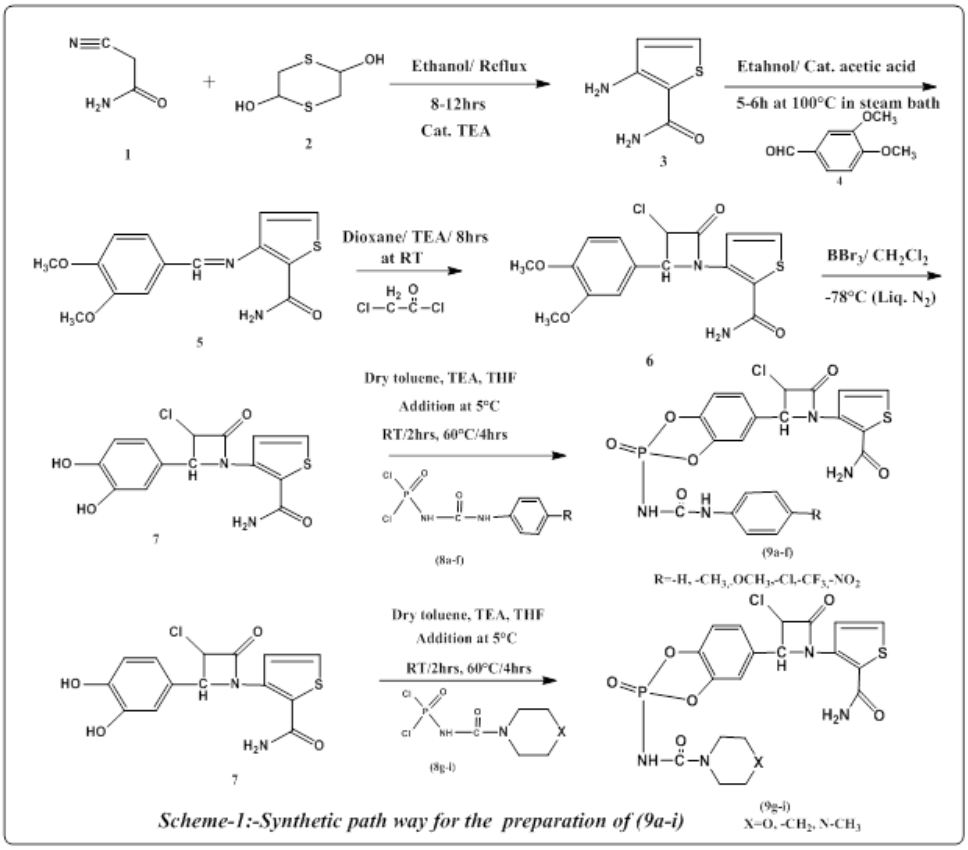
Heterocycles possessing azetidin-2-one ring were found to show various types of biological activities.[3] Azetidinones are very important class of compounds possessing wide range of biological activities such as antimicrobial[4], pesticidal[4], antitumor[5], antitubercular[6], anticancer[7], cytotoxic[8], enzyme inhibitors[9], elastase inhibitors[10] and cholesterol absorption inhibitors.[9] Many β-lactam drugs had been reported in the literature. In the present studies, we have reported a molecular frame work, which possess ureido/carboxamide, azetidinone-thiophene-organophosphorus compounds. The different 3-(3-chloro)-2-(2-oxido)-(4-substituted phenyl)-ureido-benzo[*d*][1,3,2]dioxaphosphol-5-yl-4-oxoazetidin-1-yl) thiophene-2-carboxamides **(9a-f)** and carbamoyl thiophene- chloro-4-oxoazetidin-dioxaphosphol Morpholine/Piperidine/4-methyl piperizine-carboxamides **(9g-i)** 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. 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 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.

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



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

Synthesis of 3-aminothiophene-2-carboxamide (3)[11],[12]

A solution of 2-cyanoacetamide (**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. 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 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).**

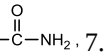
IR (KBr pellet), ν, cm^{-1}: Characteristic bands around 3400 and 3420 str. of –NH_2 of amide group, 3330, 3345 str. of amine group, 3020 str. of Aromatic protons of thiophene ring, 1670 str. of $-\overset{O}{\overset{\|}{C}}-$ of amide –I band and 1450, 675 characteristic bands of thiophene ring.

^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 7.80 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)[13]: 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. The progress of the reaction was monitored by TLC analysis.

IR (KBr pellet), ν, cm^{-1}: Characteristic bands around 3400 and 3420 str. of –NH_2 of amide group, 3040 str. of Aromatic protons of Benzene ring and thiophene ring, 1670 str. of  of amide –I band, 1620 str. of -CH=N- of azo methine, 1450, 675 characteristic bands of thiophene ring and 1050 $\delta_{\text{-c-o-c-}}$ of aromatic ether.

^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 3.80 s 6H two –OCH_3 groups, 7.80 s,2H,-NH_2 of amide group,  , 7.1-7.30 m 5H C_6H_3 of Benzene ring and 2H of thiophene ring and 8.30 s H C-H of azo methine group).

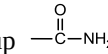
Synthesis of 3-(3-chloro-2-(3,4-dimethoxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide(6)[14],[15]
Monochloro acetyl chloride(0.025moles) was added drop wise to the compound **(5,** 0.02moles**)** and triethyl amine in dioxane (25ml) at room temperature. The mixture was stirred for 8 hours and left at room temperature for 3-days. Pour the contents on crushed ice to afford 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◦C with a yield of 70%, 0.014moles. The separated solid was identified as 3-(3-chloro-2-(3,4-dimethoxyphenyl) -4-oxoazetidin-1-yl)thiophene-2-carboxamide**(6).** The progress of the reaction was monitored by TLC analysis.

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

^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 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 group 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)[16]: A solution of 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 PH 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-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl) thiophene-2-carboxamide**(7).** The m.p. of **(7)** was found to be 139-141°C with a yield of 60%, 0.012 moles. The progress of the reaction was monitored by TLC analysis.

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

^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 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/morpholinyl/piperidinyl/N-methyl piperizinyl-carbamido-phosphoric acid dichlorides (8a-i)[17],[18]: 4-substituted phenyl/morpholinyl/piperidinyl/N-methyl piperizinyl-carbamido-phosphoric acid dichlorides **(8a-i)** were synthesized as reported in the literature.

General procedure for the synthesis of (4-substituted phenyl)-ureido-benzodioxo phosphol-oxoazetidin-thiophene carboxamides (9a-f) and Morpholine/Piperidine/4-methyl piperizine carboxamides (9g-i)[19]
A solution of (phenyl carbamoyl)phosphoramidic dichloride(**8a**, 0.02moles) in 25ml of dry toluene was added drop wise over a period of 20 minutes to a stirred solution of 3-(3-chloro-2-(3,4-dihydroxyphenyl)-4-oxoazetidin-1-yl)thiophene-2-carboxamide(**7**, 0.02moles) and triethylamine (0.04moles) in 30ml of dry toluene and 10ml of tetrahydrofuran at 5°C. After completion of the 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^{o}C and maintained for 4 hours 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 of 3-(3-chloro-2-(2-oxido-2-(3-phenylureido) benzo[d][1,3,2] dioxaphosphol-5-yl) -4-oxoazetidin-1-yl)thiophene-2-carboxamide **(9a)**. The m.p. was found to be 117-119^{o}C with a yield of 70%, 0.014moles.

The similar procedure was adopted to synthesize **(9b-f)** by the reaction between **(7)** with *p*-tolyl carbamoyl phosphoramidic dichloride**(8b)**, 4-methoxy phenyl carbamoyl phosphoramidic dichloride**(8c),** 4-chloro phenyl carbamoyl phosphoramidic dichloride**(8d)**, 4-(trifluoromethyl)phenyl carbamoyl phosphoramidic dichloride**(8e)** and 4-nitrophenyl carbamoyl phosphoramidic dichloride**(8f).**

The reaction between **(7)** with (morpholine-4-carbonyl) phosphoramidic dichloride **(8g),** (piperidine-1-carbonyl) phosphoramidic dichloride**(8h)** and 4-methyl piperazine-1-carbonyl phosphoramidic dichloride**(8i)** afforded **(9g-i)** respectively.

RESULTS AND DISCUSSION

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

9a: Yield: 70%. m.p:117-119˚C. Anal. Found for $C_{21}H_{16}ClN_4O_6$ P S(%): C 47.63, H 3.19, Cl 6.72, N 10.65, P 5.86 and S 6.05. IR (KBr pellet), ν, cm^{-1}: 3400 & 3450 -NH_2 of amide group, 3320 of P-NH, 3040 aromatic protons of Benzene ring and thiophene ring, 1690 —C(=O)— of azetidinone, 1670 —C(=O)— of amide –I Band, 1660 –CO- of ureido group, 1450-675 characteristic bands of thiophene ring, 1415 C-N of azetidin-2-one ring, 1250 P=O, 954 P-O, 670 C-Cl . ^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 5.05 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to –Cl, 8.90 s 1H –NH- group of urea attached to P=O, 7.80 s 2H -NH_2 group of amide group —C(=O)—NH$_2$, 6.9-7.3 m 10H C_6H_3, C_6H_5 and two thiophene protons and 8.70 s 1H –NH- group of urea attached to benzene ring. ^{13}C-NMR (δ ppm): 112.3, 134.8, 118.0, 145.0, 162.2, 62.0, 68.4, 137.5, 114.0, 145.0, 143.2, 117.1, 119.6, 152.0, 139.4, 121.6, 128.9, 128.0 and 162.3 corresponding to 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}, $C_{15,}$ $C_{16,}$ $C_{17,}$ $C_{18,}$ $C_{19,}$ C_{20} and C_{21} respectively. The molecule has 19 non-equivalent carbon atoms. P^{31}-NMR (δ, ppm): -6.9. Mass (m/z %): 518 $\overline{M}^{\cdot+}$, 520 M^{+2}.

9b: Yield: 70%. m.p:132-134˚C. Anal. Found for $C_{22}H_{18}ClN_4O_6$ P S(%): C 48.6, H 3.32, Cl 6.54, N 10.33, P 5.71 and S 5.88. IR (KBr pellet), ν, cm^{-1}: 3390 & 3410 -NH_2 of amide group, 3315 of P-NH, 3025 aromatic protons of Benzene ring and thiophene ring, 1685 —C(=O)— of azetidinone, 1665 —C(=O)— of amide –I Band, 1655 –CO- of ureido group, 1450-675 characteristic bands of thiophene ring, 1410 C-N of azetidin-2-one ring, 1245 P=O, 950 P-O, 662 C-Cl,. ^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 2.40 s 3H of methyl group, 5.05 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to –Cl, 8.90 s 1H –NH- group of urea attached to P=O, 7.80 s 2H -NH_2 group of amide group —C(=O)—NH$_2$, 6.9-7.3m 9H C_6H_3,C_6H_4 and two thiophene protons and 8.70 s 1H –NH- group of urea attached to benzene ring. P^{31}-NMR (δ, ppm): -7.5.

9c: Yield: 70%. m.p:109-111˚C. Anal. Found for $C_{22}H_{18}ClN_4O_7PS$ (%): C 47.16, H 3.22, Cl 6.35, N 10.02, P 5.54 and S 5.72. IR (KBr pellet), ν, cm^{-1}: 3395 & 3415 -NH_2 of amide group, 3310 of P-NH, 3040 aromatic protons of Benzene ring and thiophene ring, 1680 —C(=O)— of azetidinone, 1660 —C(=O)— of amide –I Band, 1650 –CO- of ureido group, 1450-675 characteristic bands of thiophene ring, 1415 C-N of azetidin-2-one ring, 1254 P=O, 958 P-O, 667 C-Cl. ^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 3.80 s 3H of –OCH_3 group, 5.05 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to –Cl, 8.90 s 1H –NH- group of urea attached to P=O, 6.9-7.3m 9H C_6H_3,C_6H_4 and two thiophene protons, 7.80 s 2H -NH_2 group of amide group —C(=O)—NH$_2$ and 8.70 s 1H –NH- group of urea attached to benzene ring. P^{31}-NMR (δ, ppm): -7.8.

9d: Yield: 70%. m.p:142-144˚C. Anal. Found for $C_{21}H_{15}Cl_2N_4O_6PS$ (%): C 44.68, H 2.66, Cl 12.62, N 9.94, P 5.49 and S 5.67. IR (KBr pellet), ν, cm^{-1}: 3405 & 3420 -NH_2 of amide group, 3330 of P-NH, 3035 aromatic protons of Benzene ring and thiophene ring, 1695 —C(=O)— of azetidinone, 1675 —C(=O)— of amide –I Band, 1670 –CO- of ureido group, 1450-675 characteristic bands of thiophene ring, 1420 C-N of azetidin-2-one ring, 1256 P=O, 956 P-O, 675 C-Cl. ^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 5.05 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to –Cl, 8.90 s 1H –NH- group of urea attached to P=O, 7.80 s 2H -NH_2 group of amide group —C(=O)—NH$_2$, 7.00-7.30 m 9H C_6H_3, C_6H_4 and two thiophene protons and 8.70 s 1H –NH- group of urea attached to benzene ring.

P^{31}-NMR (δ, ppm): -7.3.

9e: Yield: 70%. m.p:128-130˚C. Anal. Found for $C_{22}H_{15}ClF_3N_4O_6P$ S (%): C 44.13, H 2.51, Cl 5.94, N

9.36, P 5.17 and S 5.35. IR (KBr pellet), ν, cm^{-1}: 3420 & 3440 $-NH_2$ of amide group, 3335 of P-NH, 3030 aromatic protons of Benzene ring and thiophene ring, 1705 —C(=O)— of azetidinone, 1680 —C(=O)— of amide –I Band, 1675 –CO- of ureido group, 1450-675 characteristic bands of thiophene ring, 1420 C-N of azetidin-2-one ring, 1260, P=O, 964 P-O, 682 C-Cl. ^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 5.05 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to –Cl, 8.90 s 1H –NH- group of urea attached to P=O, 7.80 s 2H $-NH_2$ group of amide group —C(=O)—NH_2, 7.00-7.30 m 9H C_6H_3, C_6H_4 and two thiophene protons and 8.70 s 1H –NH- group of urea attached to benzene ring.

P^{31}-NMR (δ, ppm): -8.1.

9f: Yield: 75%. m.p:157-159°C. Anal. Found for $C_{21}H_{15}ClN_5O_8PS$ (%): C 43.83, H 2.61, Cl 6.19, N 12.19, P 5.39 and S 5.57. IR (KBr pellet), ν, cm^{-1}: 3430 & 3450 $-NH_2$ of amide group, 3340 of P-NH, 3040 aromatic protons of Benzene ring and thiophene ring, 1710 —C(=O)— of azetidinone, 1690 —C(=O)— of amide –I Band, 1680 –CO- of ureido group, 1450-675 characteristic bands of thiophene ring, 1425 C-N of azetidin-2-one ring, 1270 P=O, 974 P-O, 687 C-Cl. ^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 5.05 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to –Cl, 8.90 s 1H –NH- group of urea attached to P=O, 7.80 s 2H $-NH_2$ group of amide group —C(=O)—NH_2, 7.00-7.4 m 9H C_6H_3, C_6H_4 and two thiophene protons and 8.70 s 1H –NH- group of urea attached to benzene ring.

P^{31}-NMR (δ, ppm): -8.6.

9g: Yield: 65%. m.p:103-105°C. Anal. Found for $C_{19}H_{18}ClN_4O_7PS$ (%): C 43.6, H 3.45, Cl 6.81, N 10.73, P 5.93 and S 6.14. IR (KBr pellet), ν, cm^{-1}: 3410 & 3425 $-NH_2$ of amide group, 3335 of P-NH, 3045 aromatic protons of Benzene ring and thiophene ring, 1695 —C(=O)— of azetidinone, 1675 —C(=O)— of amide –I Band, 1660 –CO- of ureido group, 1450-675 characteristic bands of thiophene ring, 1420 C-N of azetidin-2-one ring, 1260 P=O, 964 P-O, 680 C-Cl. ^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 2.86-3.6 m 8H of morpholine ring, 5.05 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to –Cl, 8.90 s 1H –NH- group of urea attached to P=O, 7.80 s 2H $-NH_2$ group of amide group —C(=O)—NH_2 and 7.00-7.3 m 5H C_6H_3 and two thiophene protons. P^{31}-NMR (δ, ppm): -7.4.

9h: Yield: 60%. m.p:114-116°C. Anal. Found for $C_{20}H_{20}ClN_4O_6PS$ (%): C 46.12, H 3.84, Cl 6.84, N 10.77, P 5.97 and S 6.15. IR (KBr pellet), ν, cm^{-1}: 3410 & 3425 $-NH_2$ of amide group, 3330 of P-NH, 3050 aromatic protons of Benzene ring and thiophene ring, 1685 —C(=O)— of azetidinone, 1665 —C(=O)— of amide –I Band, 1660 –CO- of ureido group, 1450-675 characteristic bands of thiophene ring, 1410 C-N of azetidin-2-one ring, 1250 P=O, 954 P-O, 675 C-Cl. ^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 1.60-2.80 m 10H of piperidine, 5.05 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to –Cl, 8.90 s 1H –NH- group of urea attached to P=O, 7.80 s 2H $-NH_2$ group of amide group —C(=O)—NH_2 and 7.00-7.3 m 5H C_6H_3 and two thiophene protons. P^{31}-NMR (δ, ppm): -7.7.

9i: Yield: 65%. m.p:121-123°C. Anal. Found for $C_{20}H_{21}ClN_5O_6PS$ (%): C 44.78, H 3.92, Cl 6.64, N 13.12, P 5.79 and S 6.97. IR (KBr pellet), ν, cm^{-1}: 3405 & 3420 $-NH_2$ of amide group, 3330 of P-NH, 3055 aromatic protons of Benzene ring and thiophene ring, 1690 —C(=O)— of azetidinone, 1670 —C(=O)— of amide –I Band, 1665 –CO- of ureido group, 1450-675 characteristic bands of thiophene ring, 1420 C-N of azetidin-2-one ring, 1250 P=O, 956 P-O, 680 C-Cl. ^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 2.23 s 3H N-CH_3 group of piperazine, 2.95-3.10 m 8H CH_2 group of piperazine, 5.05 d 1H -CH- of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to –Cl, 8.90 s 1H –NH- group of urea attached to P=O, 7.80 s 2H $-NH_2$ group of amide group —C(=O)—NH_2 and 7.00-7.3 m 5H C_6H_3 and two thiophene protons. P^{31}-NMR (δ, ppm): -7.6

Biological activity: The antimicrobial activity[20] of chemical compound is influenced by physical and biological characteristics.[21] It is well established that physiological activity is a function of the chemical structure of compound.[22] Heterocyclic organic compounds containing phosphorus, oxygen, Nitrogen or sulfur in the ring system are expected to be more active due to the presence of hetero atoms.[23],[24]

In view of this, the synthesized new organophosphorus heterocyclic compounds have been tested for the antimicrobial activity.

Antibacterial activity: The antibacterial activity[25] of 3-(3-chloro)-2-(2-oxido)-(4-substituted phenyl)ureido-benzo[*d*][1,3,2] dioxaphosphol-5-yl-4-oxoazetidin-1-yl)thiophene-2-carboxamides **(9a-f)** and carbamoyl thiophene- chloro-4-oxoazetidin-dioxaphosphol Morpholine/Piperidine/4-methyl piperizine-carboxamides **(9g-i)** were screened against the Staphylococcus aureus (gram positive), Bacillus cereus, Escherichia coli (gram negative) and Pseudomonas aeruginosa organisms. Most of the compounds exhibit moderate to good antibacterial activity against both

bacteria under present investigations. The substituents nitro **(9f),** trifluoro methyl **(9e)** and chloro **(9d)** showed more activity than the other substituted compounds in the first series **(9a-f)**. In the second series **(9g-i)**, the substituent piperidine **(9h)** showed more activity than the other substituted compounds. The antibacterial activity of **(9a-f)** was shown in the **Table-1** and the antibacterial activity of **(9g-i)** was shown in the **Table-2** and **Fig-1.** Here ***Amoxicillin*** is used as reference compound to compare the activity.

The order of anti-bacterial activity was found to be
9f>9e>9d>9b>9a>9c in the first series of compounds (9a-f)
9h>9g>9i in the second series of compounds (9g-i)

Table 1: Antibacterial activity (Diameter zone of inhibition in mm) of Compounds (9a-f) (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	09	07	07	07
2	9b	10	08	08	07
3	9c	07	06	06	05
4	9d	11	09	10	09
5	9e	15	11	13	12
6	9f	17	12	15	13
Amoxicillin		21	27	24	22

Table 2: Antibacterial activity (Diameter zone of inhibition in mm) of Compounds (9g-i) (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	9g	15	13	13	11
2	9h	18	16	15	14
3	9i	14	10	12	09
Amoxicillin		21	27	24	22

Antifungal activity: Antifungal activity of final compounds 3-(3-chloro)-2-(2-oxido)-(4-substituted phenyl)ureido-benzo[*d*][1,3,2]dioxaphosphol-5-yl-4-oxoazetidin-1-yl)thiophene-2-carboxamides **(9a-f)** and carbamoyl thiophene-chloro-4-oxoazetidin-dioxaphosphol Morpholine/Piperidine/4-methyl piperizine-carboxamides **(9g-i)** were screened against the Aspergillus niger, Candida albicans.[26] The substituents nitro **(9f),** trifluoro methyl**(9e)** and chloro **(9d)** showed more activity than other substituted compounds in the first series**(9a-f)**. In the second series **(9g-i)**, the substituent piperidine **(9h)** showed more activity than other substituted compounds. The antifungal activity of **(9a-f)** was shown in the **Table-3** and the antifungal activity of **(9g-i)** was shown in the **Table-4** and **Fig-2.** Here ***Ketoconazole*** is used as reference compound to compare the activity.

The order of anti-fungal activity was found to be
9f>9d>9e>9b>9a>9c in the first series of compounds (9a-f)
9h>9i>9g in the second series of compounds (9g-i)

Table 3: Antifungal activity ((Diameter zone of inhibition in mm) of Compounds (9a-f) (250µg/ml)

S.No	Comp	Zone of inhibition (mm)	
		Aspergillius niger NCCS 1196	Candida albicans NCCS 3471
1	9a	12	11
2	9b	13	12
3	9c	10	08
4	9d	16	14
5	9e	14	13
6	9f	17	15
Ketoconazole		22	25

Table 4: Antifungal activity (Diameter zone of inhibition in mm) of Compounds (9g-i) (250µg/ml)

S.No	Comp	Zone of inhibition (mm)	
		Aspergillius niger NCCS 1196	Candida albicans NCCS 3471
1	9g	14	13
2	9h	18	16
3	9i	15	14
Ketoconazole		22	25

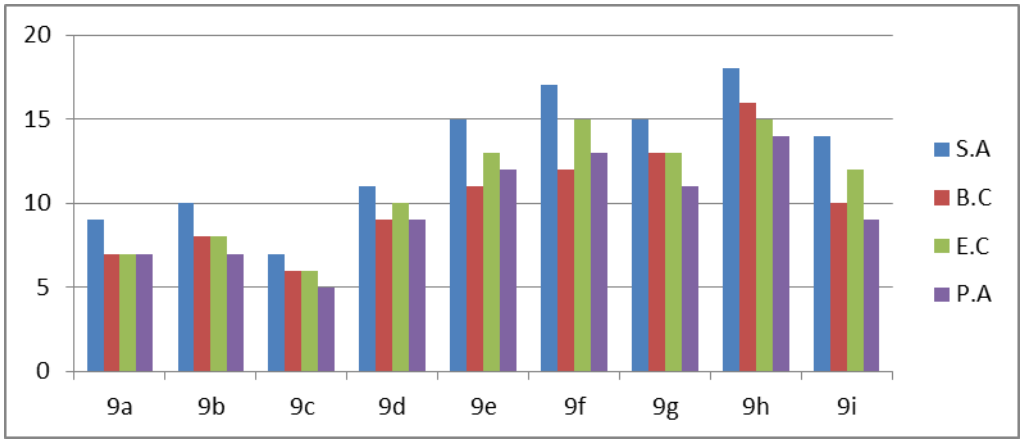


Fig. 1. Antibacterial activity of compounds (9a-i)

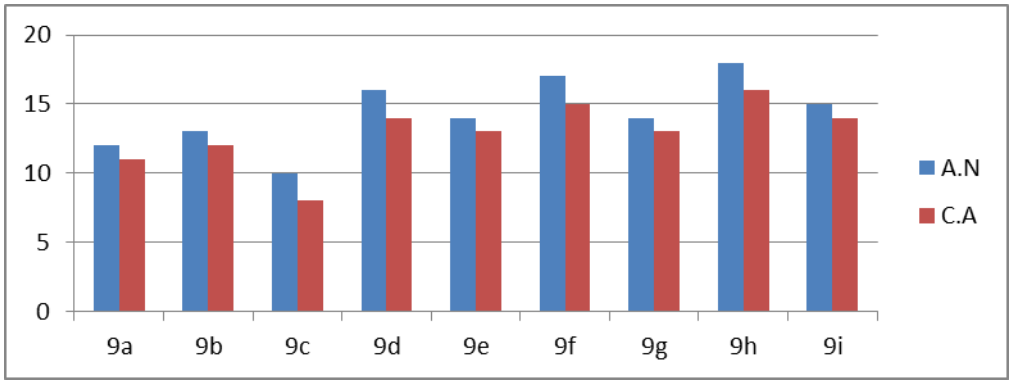
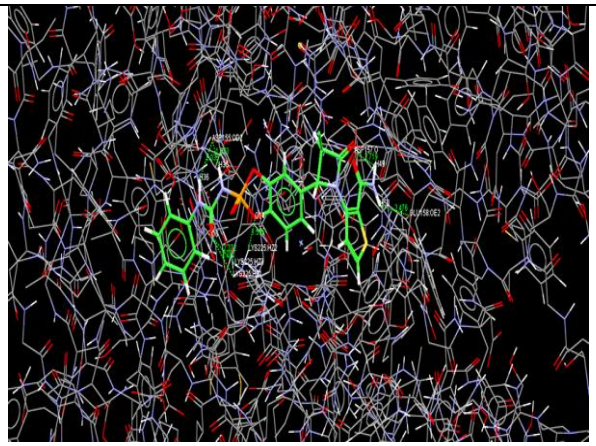


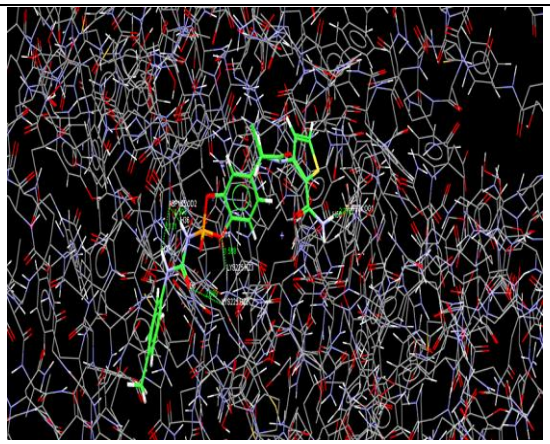
Fig. 2. Antifungal activity of compounds (9a-i)

Docking Studies of the compounds (9a-i): Docking[27] was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). The docking studies of (**9a-i)** were carried out as model compounds on ***Mycobacterium Tuberculosis Catalase-Peroxidase(Kat G)***.[28] The docking ligands were found to have some interactions between an oxygen atom of the ligands and ***Mycobacterium Tuberculosis Catalase-Peroxidase(Kat G)*** enzyme. The results pertaining to Docking studies were shown in the **Table-5- Table-6** 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-i).** The hydrogen bondings were noticed between Lysine(225), Lysine (226), Aspartic acid (157), Aspartic acid (153), Aspartic acid (155), Aspartic acid (161), Glutamic acid (158), Threonine (224), glutamic acid(158), and Serine (16). The order of protein-ligand vanderwaals score of interaction was found to be **9d>9a>9e>9i>9c=9f>9b>9g>9h** 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 **9d>9a>9e>9i>9c=9f>9b>9g>9h.** According to gold score fitness value ligand **9d** exhibits high binding activity with the protein and ligand **9h** showed the least binding activity with the protein. Comparative Gold Score fitness values for **(9a-i)** were shown in **Fig.3.**

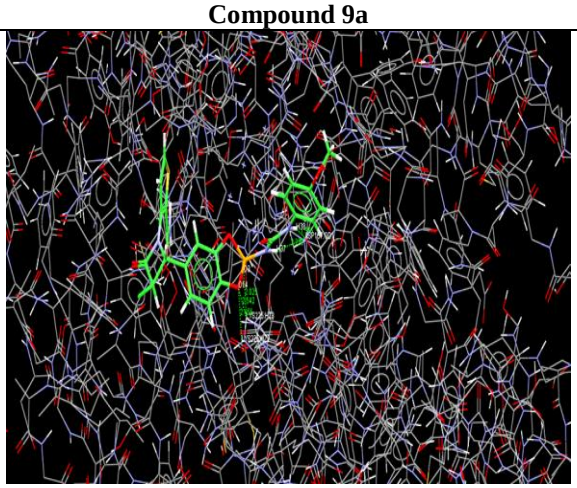
In Gold score evaluation of docking studies, electronic interactions, bonding interactions, steric interaction and conformations of proteins and docked ligand play significant role. However, in the evaluation of antimicrobial studies, electronic factors of the substituents play a significant role.



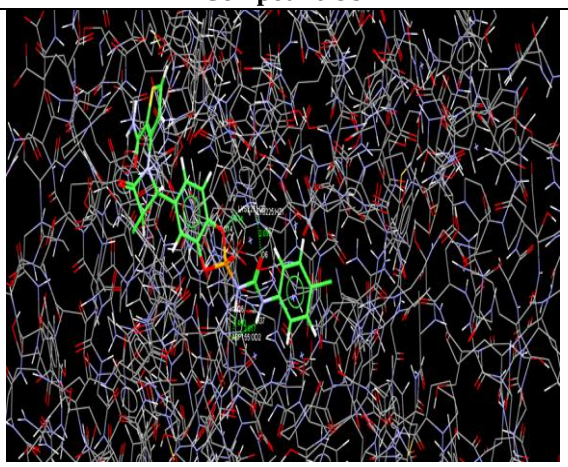
Compound 9a



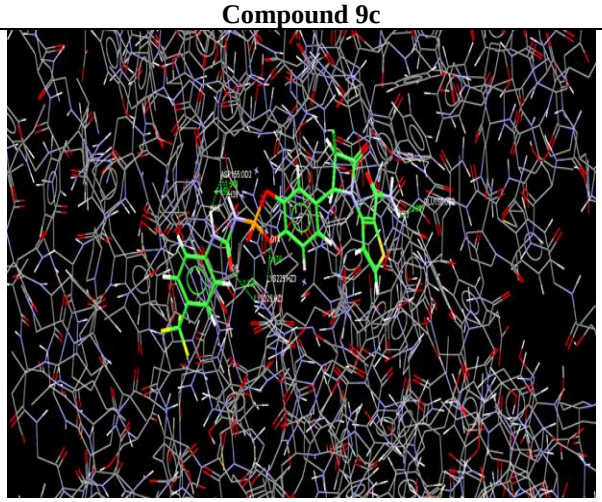
Compound 9b



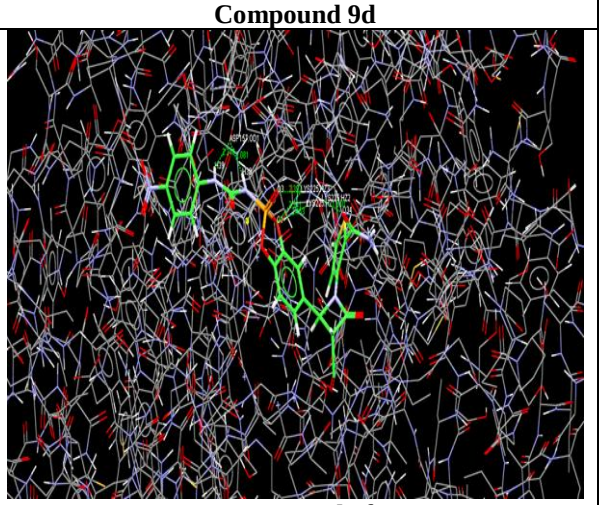
Compound 9c



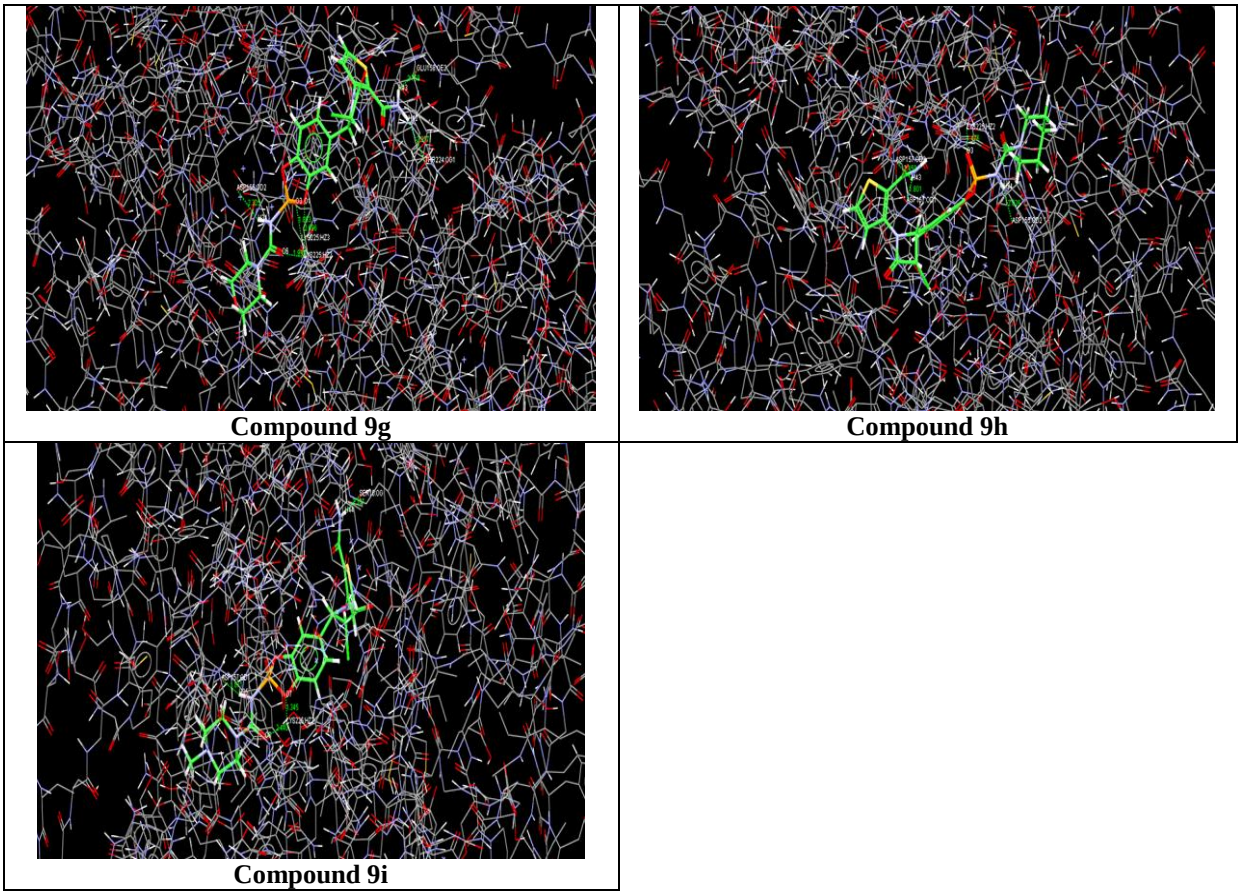
Compound 9d



Compound 9e



Compound 9f



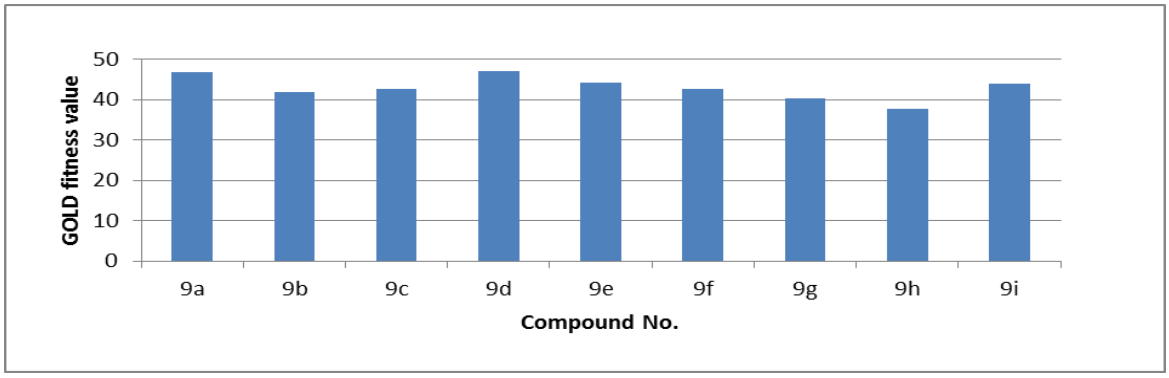


Fig. 3: Comparative Gold Score Fitness values for Compounds (9a-i)

Table 5: Docking results of (9a-g) on Mycobacterium Tuberculosis Catalase-Peroxidase (KatG)

Comp	R/X	Fitness	S(Hb_ext)	S(vdw_ext)	S(Hb_int)	S(vdw_int)
9a	H	46.91	12.47	26.20	0.00	-1.59
9b	CH_3	41.84	11.99	23.15	0.00	-1.99
9c	OCH_3	42.51	11.60	24.26	0.00	-2.45
9d	Cl	46.95	10.27	27.45	0.00	-1.07
9e	CF_3	44.14	8.30	27.52	0.00	-2.00
9f	NO_2	42.51	18.38	22.52	0.00	-6.84
9g	O	40.17	7.30	24.55	0.00	-0.89
9h	CH_2	37.75	14.98	17.11	0.00	-0.75
9i	N-CH_3	44.02	6.48	29.34	0.00	-2.74

Table 6: Hydrogen bonding interactions of Compounds (9a-i) with Mycobacterium Tuberculosis Catalase-Peroxidase (KatG)

Comp No	R/X	No of 'H' bonds	Compounds		Bond Length (A^o)	Fitness
			Protein	Atoms		
9a	H	5	LYS225:HZ2 LYS225:HZ3 LYS225:HZ1 ASP155:OD2 ASP155:OD2	O14 O6 O6 H35 H36	1.555 2.372 2.392 1.573 2.033	46.91
9b	CH_3	4	ASP155:OD2 ASP155:OD2 LYS225:HZ2 LYS225:HZ3	H36 H37 O8 O14	1.503 2.121 2.689 1.588	41.83
9c	OCH_3	5	ASP157:OD1 ASP157:OD1 LYS225:HZ3 LYS225:HZ3 LYS225:HZ2	H38 H37 O3 O14 O14	1.566 2.228 2.025 2.542 2.456	42.51
9d	Cl	4	ASP155:OD2 ASP155:OD2 LYS225:HZ1 LYS225:HZ2	H36 H37 O6 O14	1.460 2.617 2.091 1.434	46.94
9e	CF_3	5	ASP155:OD2 ASP155:OD2 LYS225:HZ3 LYS225:HZ1 GLU158:O52	H46 H39 O11 O8 H53	1.652 1.569 1.678 2.238 3.382	44.14
9f	NO_2	6	ASP153:OD1 ASP153:OD1 LYS225:HZ1 LYS225:HZ2 LYS225:HZ3 LYS225:HZ3	H38 H39 O1 O34 O3 O1	2.081 2.248 2.463 1.640 2.397 2.241	42.51
9g	O	6	ASP155:OD2 LYS225:HZ2 LYS225:HZ3 LYS225:HZ2 GLU158:OEZ THR224:OG1	H36 O6 O3 O1 H43 H42	2.325 1.995 1.863 2.896 2.159 2.977	40.17
9h	CH_2	4	ASP157:OD2 ASP161:OD1 ASP135:OD2 LYS225:HZ2	H43 H43 H34 O3	2.450 1.801 2.026 1.476	37.76
9i	N-CH_3	4	SER16:OG LYS226:HZ2 LYS226:HZ2 ASP157:OD1	H44 O7 O6 H35	2.307 1.345 2.440 1.871	44.08

4. CONCLUSION

In current research work, few anologues of (4-substituted phenyl) -ureido-benzodioxophosphol-oxoazetidin-thiophene carboxamides **(9a-f)** and Morpholine/Piperidine/4-methyl piperizine carboxamides **(9g-i)** were successfully prepared and characterized. Biological activity and docking studies of these compounds were also conducted.

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