

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)-CARBOXAMIDESY. N. Spoorthy¹, T. Sailaja Rani*¹, P. Vijaya Kumar² and L. K. Ravindranath³¹Research Scholar, Dept. of Chemistry, Sri Krishna Devaraya University, Anantapur, Andhra Pradesh, India.²Lecturer in Chemistry, Govt. College (Men), Anantapur, Andhra Pradesh, India.³Professor, Dept. of Chemistry, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India.***Corresponding Author: T. Sailaja Rani**

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Article Received on 23/07/2021

Article Revised on 12/08/2021

Article Accepted on 02/09/2021

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 piperazine-carboxamides (**9g-i**) were synthesized by condensing (**7**) with Morpholinyl/Piperidinyl/N-methyl piperiziny-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-carboxamide (**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, ¹H-NMR, ¹³C-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 piperazine-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

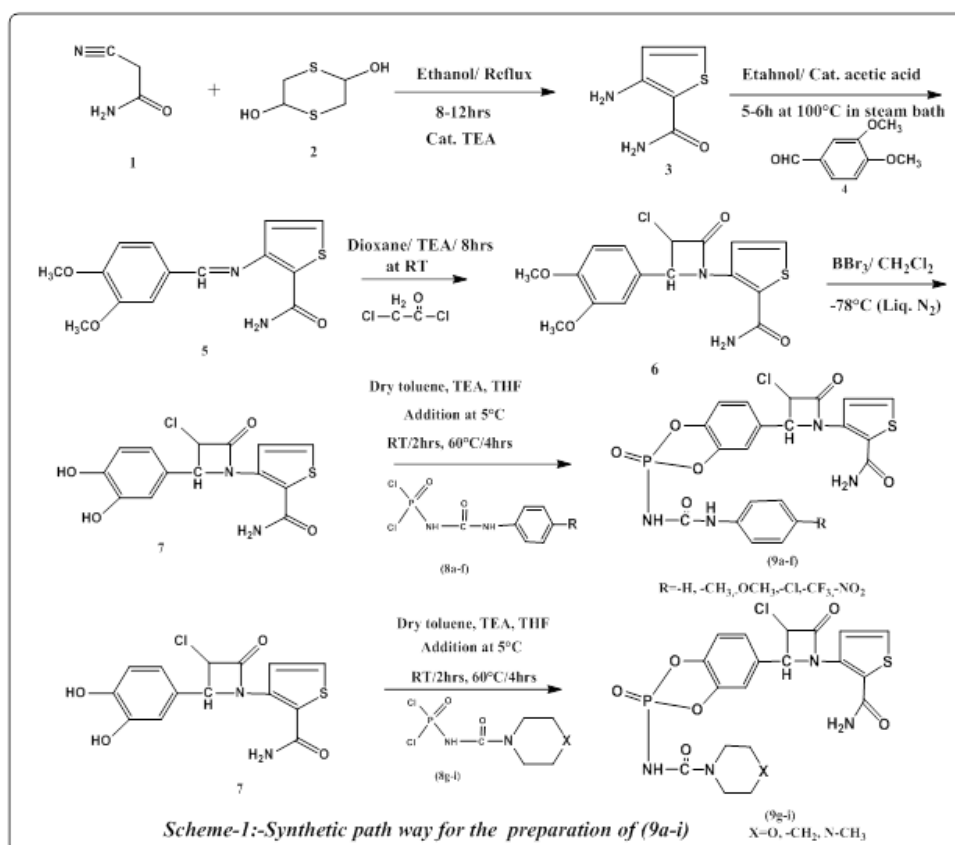
absorption inhibitors.^[9] Many β -lactam drugs had been reported in the literature. In the present studies, we have reported a molecular frame work, which possess ureido/carboxamide, azetidinone-thiophene-organophosphorus compounds. The different 3-(3-chloro)-2-(2-oxido)-(4-substituted phenyl)-ureido-benzo[d][1,3,2]dioxaphosphol-5-yl-4-oxoazetidin-1-yl)thiophene-2-carboxamides (**9a-f**) and carbamoyl thiophene-chloro-4-oxoazetidin-dioxaphosphol Morpholine/Piperidine/4-methyl piperazine-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 ^1H and ^{13}C -NMR spectra were recorded on a Varian XL-300

spectrometer operating at 400MHz for ^1H -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 (^1H 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**.



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 eluent. After the completion of reaction, the solvent was evaporated under reduced pressure and the reaction mass kept at room temperature. The isopropyl alcohol was added and maintained the reaction mass at room temperature for 1 hour. The solid was filtered and washed; the wet material with isopropyl alcohol was dried under suction. The residue was recrystallized from 2-propanol. The m.p. of (3) was found to be 122-124°C with a yield of 75%,

0.015moles. The separated solid was identified as 3-aminothiophene-2-carboxamide (3).

IR (KBr pellet), ν , cm^{-1} : Characteristic bands around 3400 and 3420 str. of $-\text{NH}_2$ of amide group, 3330, 3345 str. of amine group, 3020 str. of Aromatic protons of

thiophene ring, 1670 str. of $-\text{C}=\text{O}$ of amide $-\text{I}$ band and 1450, 675 characteristic bands of thiophene ring.

^1H -NMR (DMSO- d_6), δ , ppm (J, Hz): 7.80 s 2H $-\text{NH}_2$ group of amide, 5.20 bs 2H $-\text{NH}_2$ 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 $-\text{NH}_2$ of amide group, 3040 str. of Aromatic protons of Benzene ring and thiophene ring, 1670 str. of $-\overset{\text{O}}{\parallel}{\text{C}}-$ of amide $-\text{I}$ band, 1620 str. of $-\text{CH}=\text{N}-$ of azo methine, 1450, 675 characteristic bands of thiophene ring and 1050 $\delta_{\text{C-O-C}}$ of aromatic ether.

$^1\text{H-NMR}$ (DMSO- d_6), δ , ppm (J, Hz): 3.80 s 6H two $-\text{OCH}_3$ groups, 7.80 s, 2H, $-\text{NH}_2$ of amide group, $-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}_2$, 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 $-\text{NH}_2$ of amide group, 3040 str. of aromatic protons of thiophene ring, 1690 str. of $>\text{C}=\text{O}$ group of azetidinone, 1670 str. of $-\overset{\text{O}}{\parallel}{\text{C}}-$ of amide $-\text{I}$ 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.

$^1\text{H-NMR}$ (DMSO- d_6), δ , ppm (J, Hz): 3.80 s, 6H, two $-\text{OCH}_3$ groups, 5.10 d 1H $-\text{CH}-$ of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone

ring, 6.20 s 2H $-\text{NH}_2$ group of amide group $-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}_2$ 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 $-\text{OH}$, 3400 and 3420 str. of amide $-\text{NH}_2$, 3040 str. of aromatic protons of Benzene and thiophene ring, 1690 str. of $>\text{C}=\text{O}$ group of azetidinone, 1670 str. of $-\overset{\text{O}}{\parallel}{\text{C}}-$ of amide $-\text{I}$ Band, 1450 & 650 characteristic bands of thiophene ring, 1415 str. of C-N of azetidin-2-one ring and 670 str. of C-Cl.

$^1\text{H-NMR}$ (DMSO- d_6), δ , ppm (J, Hz): 5.1 d 1H $-\text{CH}-$ of azetidinone ring attached to phenyl ring, 5.60 s 2H two $-\text{OH}$ groups, 5.40 d 1H CH of azetidinone ring, 7.5 s 2H $-\text{NH}_2$ group of amide group $-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}_2$ and 6.9-7.3 m 5H C_6H_3 and 2H of thiophene ring).

Synthesis of 4-substituted phenyl/morpholinyl/piperidinyl/N-methyl piperiziny-carbamido-phosphoric acid dichlorides (8a-i**)^{[17],[18]}:** 4-substituted phenyl/morpholinyl/piperidinyl/N-methyl piperiziny-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 piperazine 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°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°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 C₂₁H₁₆ClN₄O₆ 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 -NH₂ of amide group, 3320 of P-NH, 3040 aromatic protons of Benzene ring and thiophene ring, 1690 $\text{—}\overset{\text{O}}{\parallel}\text{C—}$ of azetidinone, 1670 $\text{—}\overset{\text{O}}{\parallel}\text{C—}$ 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. ¹H-NMR (DMSO-d₆), 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 -NH₂ group of amide group $\text{—}\overset{\text{O}}{\parallel}\text{C—NH}_2$, 6.9-7.3 m 10H C₆H₃, C₆H₅ and two thiophene protons and 8.70 s 1H -NH- group of urea attached to benzene ring. ¹³C-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 C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₅, C₁₆, C₁₇, C₁₈, C₁₉, C₂₀ and C₂₁ respectively. The molecule has 19 non-equivalent carbon atoms. P³¹-NMR (δ , ppm): -6.9. Mass (m/z %): 518 $\overline{\text{M}}^+$, 520 M^{+2} .

9b: Yield: 70%. m.p:132-134°C. Anal. Found for C₂₂H₁₈ClN₄O₆ 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 -NH₂ of amide group, 3315 of P-NH, 3025 aromatic protons of Benzene ring and thiophene ring, 1685 $\text{—}\overset{\text{O}}{\parallel}\text{C—}$ of azetidinone, 1665 $\text{—}\overset{\text{O}}{\parallel}\text{C—}$ 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. ¹H-NMR (DMSO-d₆), 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 -NH₂ group of amide group $\text{—}\overset{\text{O}}{\parallel}\text{C—NH}_2$, 6.9-7.3m 9H C₆H₃, C₆H₄ and two thiophene protons and 8.70 s 1H -NH- group of urea attached to benzene ring. P³¹-NMR (δ , ppm): -7.5.

9c: Yield: 70%. m.p:109-111°C. Anal. Found for C₂₂H₁₈ClN₄O₇PS (%): 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 -NH₂ of amide group, 3310 of P-NH, 3040 aromatic protons of Benzene ring and thiophene ring, 1680 $\text{—}\overset{\text{O}}{\parallel}\text{C—}$ of azetidinone, 1660 $\text{—}\overset{\text{O}}{\parallel}\text{C—}$ 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. ¹H-NMR (DMSO-d₆), δ , ppm (J, Hz): 3.80 s 3H of -OCH₃ 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 C₆H₃, C₆H₄ and two thiophene protons, 7.80 s 2H -NH₂ group of amide group $\text{—}\overset{\text{O}}{\parallel}\text{C—NH}_2$ and 8.70 s 1H -NH- group of urea attached to benzene ring. P³¹-NMR (δ , ppm): -7.8.

9d: Yield: 70%. m.p:142-144°C. Anal. Found for C₂₁H₁₅Cl₂N₄O₆PS (%): 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 -NH₂ of amide group, 3330 of P-NH, 3035 aromatic protons of Benzene ring and thiophene ring, 1695 $\text{—}\overset{\text{O}}{\parallel}\text{C—}$ of azetidinone, 1675 $\text{—}\overset{\text{O}}{\parallel}\text{C—}$ 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. ¹H-NMR (DMSO-d₆), 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 -NH₂ group of amide group $\text{—}\overset{\text{O}}{\parallel}\text{C—NH}_2$, 7.00-7.30 m 9H C₆H₃, C₆H₄ and two thiophene protons and 8.70 s 1H -NH- group of urea attached to benzene ring.

P³¹-NMR (δ , ppm): -7.3.

9e: Yield: 70%. m.p:128-130°C. Anal. Found for C₂₂H₁₅ClF₃N₄O₆P 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 $-\text{NH}_2$ of amide group, 3335 of P-NH, 3030 aromatic protons of Benzene ring and thiophene ring, 1705 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of azetidinone, 1680 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of amide -I Band, 1675 $-\text{CO}-$ 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. $^1\text{H-NMR}$ (DMSO- d_6), δ , ppm (J, Hz): 5.05 d 1H $-\text{CH}-$ of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to $-\text{Cl}$, 8.90 s 1H $-\text{NH}-$ group of urea attached to P=O, 7.80 s 2H $-\text{NH}_2$ group of amide group $-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}_2$, 7.00-7.30 m 9H C_6H_3 , C_6H_4 and two thiophene protons and 8.70 s 1H $-\text{NH}-$ group of urea attached to benzene ring.

$\text{P}^{31}\text{-NMR}$ (δ , ppm): -8.1.

9f: Yield: 75%. m.p:157-159°C. Anal. Found for $\text{C}_{21}\text{H}_{15}\text{ClN}_5\text{O}_8\text{PS}$ (%): 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 $-\text{NH}_2$ of amide group, 3340 of P-NH, 3040 aromatic protons of Benzene ring and thiophene ring, 1710 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of azetidinone, 1690 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of amide -I Band, 1680 $-\text{CO}-$ 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. $^1\text{H-NMR}$ (DMSO- d_6), δ , ppm (J, Hz): 5.05 d 1H $-\text{CH}-$ of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to $-\text{Cl}$, 8.90 s 1H $-\text{NH}-$ group of urea attached to P=O, 7.80 s 2H $-\text{NH}_2$ group of amide group $-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}_2$, 7.00-7.4 m 9H C_6H_3 , C_6H_4 and two thiophene protons and 8.70 s 1H $-\text{NH}-$ group of urea attached to benzene ring.

$\text{P}^{31}\text{-NMR}$ (δ , ppm): -8.6.

9g: Yield: 65%. m.p:103-105°C. Anal. Found for $\text{C}_{19}\text{H}_{18}\text{ClN}_4\text{O}_7\text{PS}$ (%): 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 $-\text{NH}_2$ of amide group, 3335 of P-NH, 3045 aromatic protons of Benzene ring and thiophene ring, 1695 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of azetidinone, 1675 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of amide -I Band, 1660 $-\text{CO}-$ 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. $^1\text{H-NMR}$ (DMSO- d_6), δ , ppm (J, Hz): 2.86-3.6 m 8H of morpholine ring, 5.05 d 1H $-\text{CH}-$ of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to $-\text{Cl}$, 8.90 s 1H $-\text{NH}-$ group of urea attached to P=O, 7.80 s 2H $-\text{NH}_2$ group of amide group $-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}_2$ and 7.00-7.3 m 5H C_6H_3 and two thiophene protons. $\text{P}^{31}\text{-NMR}$ (δ , ppm): -7.4.

9h: Yield: 60%. m.p:114-116°C. Anal. Found for $\text{C}_{20}\text{H}_{20}\text{ClN}_4\text{O}_6\text{PS}$ (%): 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

$-\text{NH}_2$ of amide group, 3330 of P-NH, 3050 aromatic protons of Benzene ring and thiophene ring, 1685 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of azetidinone, 1665 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of amide -I Band, 1660 $-\text{CO}-$ 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. $^1\text{H-NMR}$ (DMSO- d_6), δ , ppm (J, Hz): 1.60-2.80 m 10H of piperidine, 5.05 d 1H $-\text{CH}-$ of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to $-\text{Cl}$, 8.90 s 1H $-\text{NH}-$ group of urea attached to P=O, 7.80 s 2H $-\text{NH}_2$ group of amide group $-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}_2$ and 7.00-7.3 m 5H C_6H_3 and two thiophene protons. $\text{P}^{31}\text{-NMR}$ (δ , ppm): -7.7.

9i: Yield: 65%. m.p:121-123°C. Anal. Found for $\text{C}_{20}\text{H}_{21}\text{ClN}_5\text{O}_6\text{PS}$ (%): 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 $-\text{NH}_2$ of amide group, 3330 of P-NH, 3055 aromatic protons of Benzene ring and thiophene ring, 1690 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of azetidinone, 1670 $-\overset{\text{O}}{\parallel}{\text{C}}-$ of amide -I Band, 1665 $-\text{CO}-$ 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. $^1\text{H-NMR}$ (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 $-\text{CH}-$ of azetidinone ring attached to phenyl ring, 5.40 d 1H CH of azetidinone attached to $-\text{Cl}$, 8.90 s 1H $-\text{NH}-$ group of urea attached to P=O, 7.80 s 2H $-\text{NH}_2$ group of amide group $-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}_2$ and 7.00-7.3 m 5H C_6H_3 and two thiophene protons. $\text{P}^{31}\text{-NMR}$ (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 piperazine-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
<i>Amoxicillin</i>		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
<i>Amoxicillin</i>		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 piperazine-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.

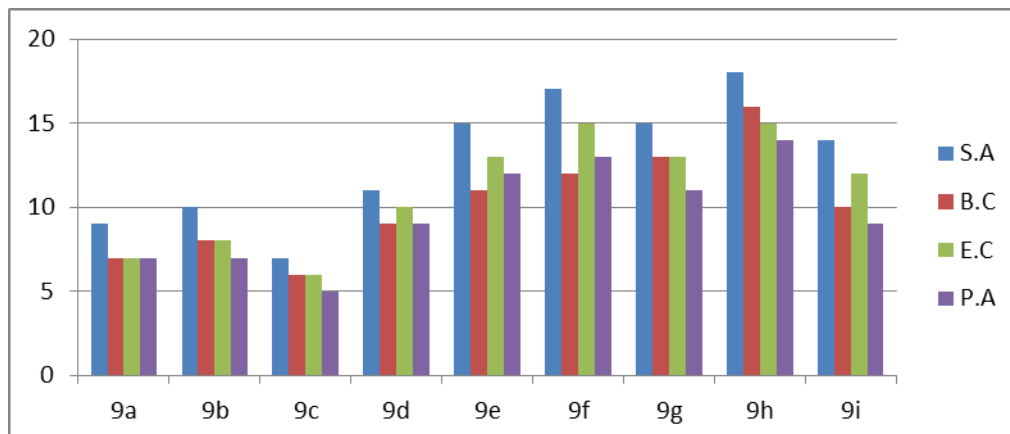
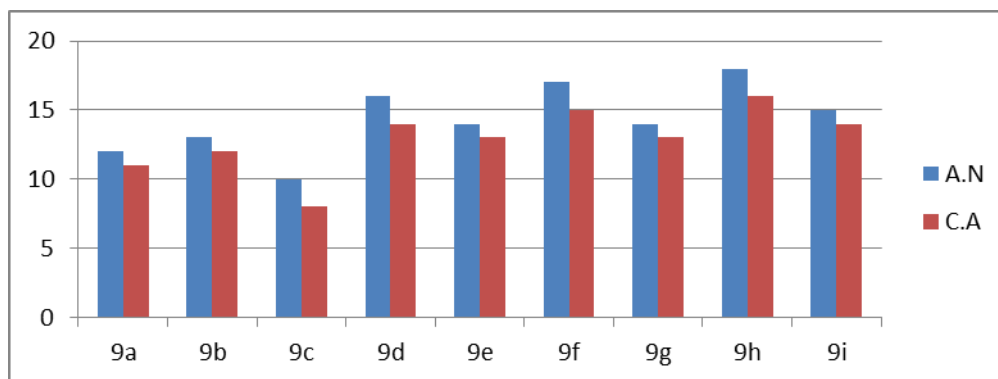
The order of anti-fungal activity was found to be **9f>9d>9e>9b>9a>9c** in the first series of compounds (**9a-f**)
9h>9i>9g in the second series of compounds (**9g-i**)

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

S.No	Comp	Zone of inhibition (mm)	
		Aspergillus 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
<i>Ketoconazole</i>		22	25

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

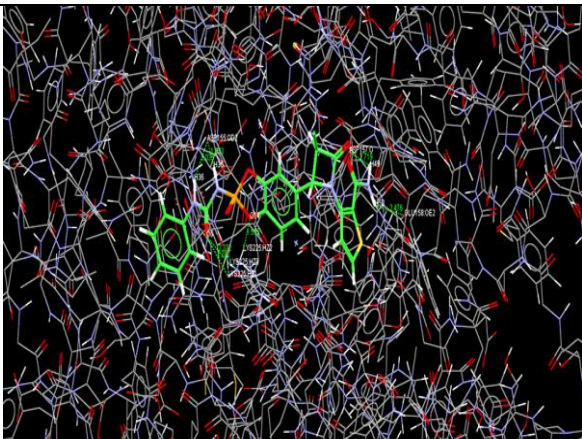
S.No	Comp	Zone of inhibition (mm)	
		Aspergillus niger NCCS 1196	Candida albicans NCCS 3471
1	9g	14	13
2	9h	18	16
3	9i	15	14
	<i>Ketoconazole</i>	22	25

**Fig. 1. Antibacterial activity of compounds (9a-i)****Fig. 2. Antifungal activity of compounds (9a-i)**

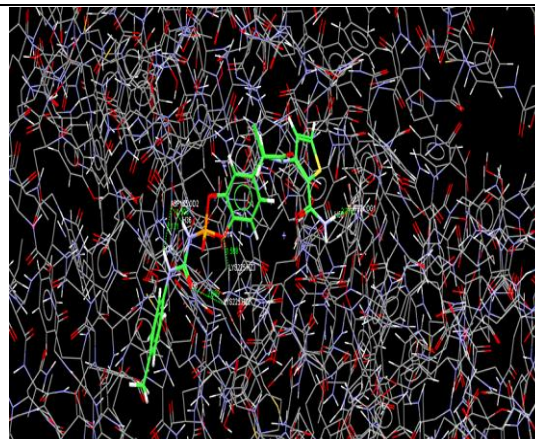
Docking Studies of the compounds (9a-i): Docking^[27] was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). The docking studies of (9a-i) were carried out as model compounds on *Mycobacterium Tuberculosis Catalase-Peroxidase(Kat G)*.^[28] 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**.

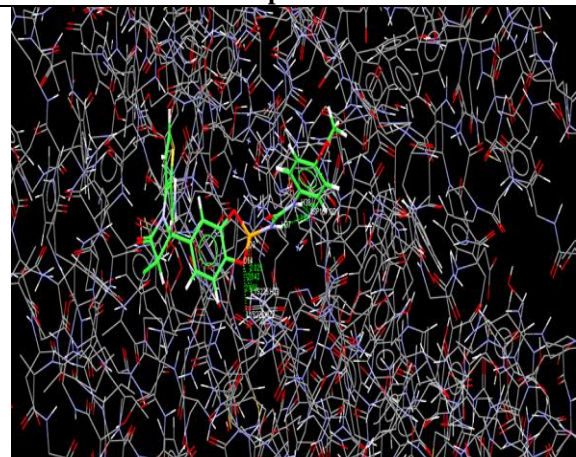
In Gold score evaluation of docking studies, electronic interactions, bonding interactions, steric interaction and conformations of proteins and docked ligand play significant role. However, in the evaluation of antimicrobial studies, electronic factors of the substituents play a significant role.



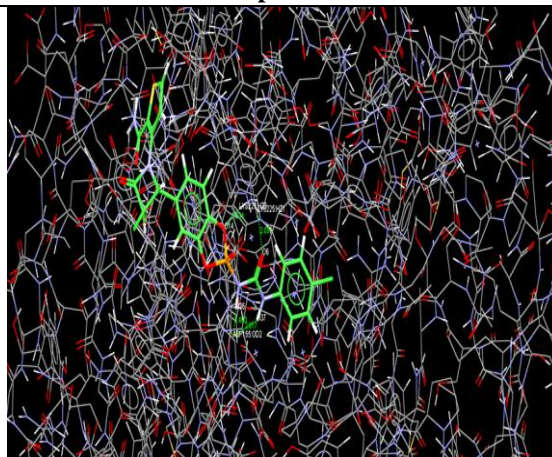
Compound 9a



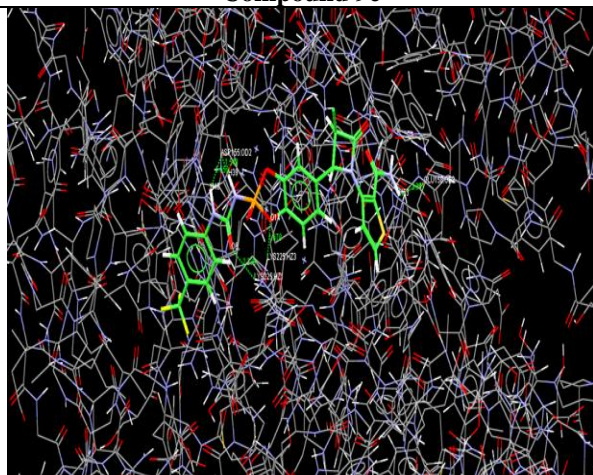
Compound 9b



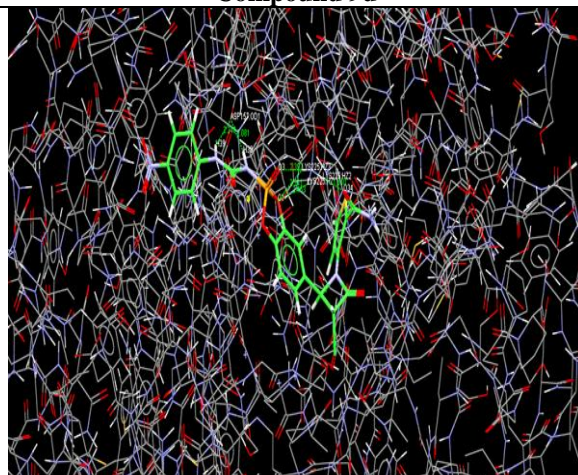
Compound 9c



Compound 9d



Compound 9e



Compound 9f

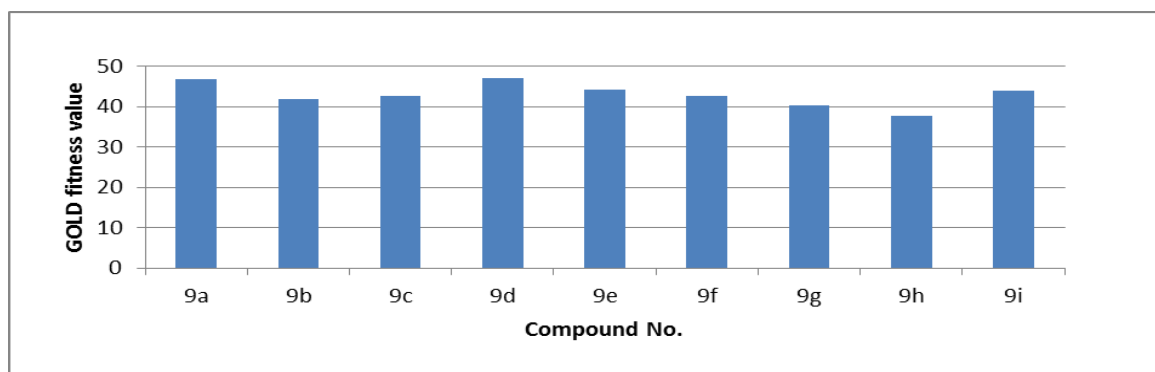
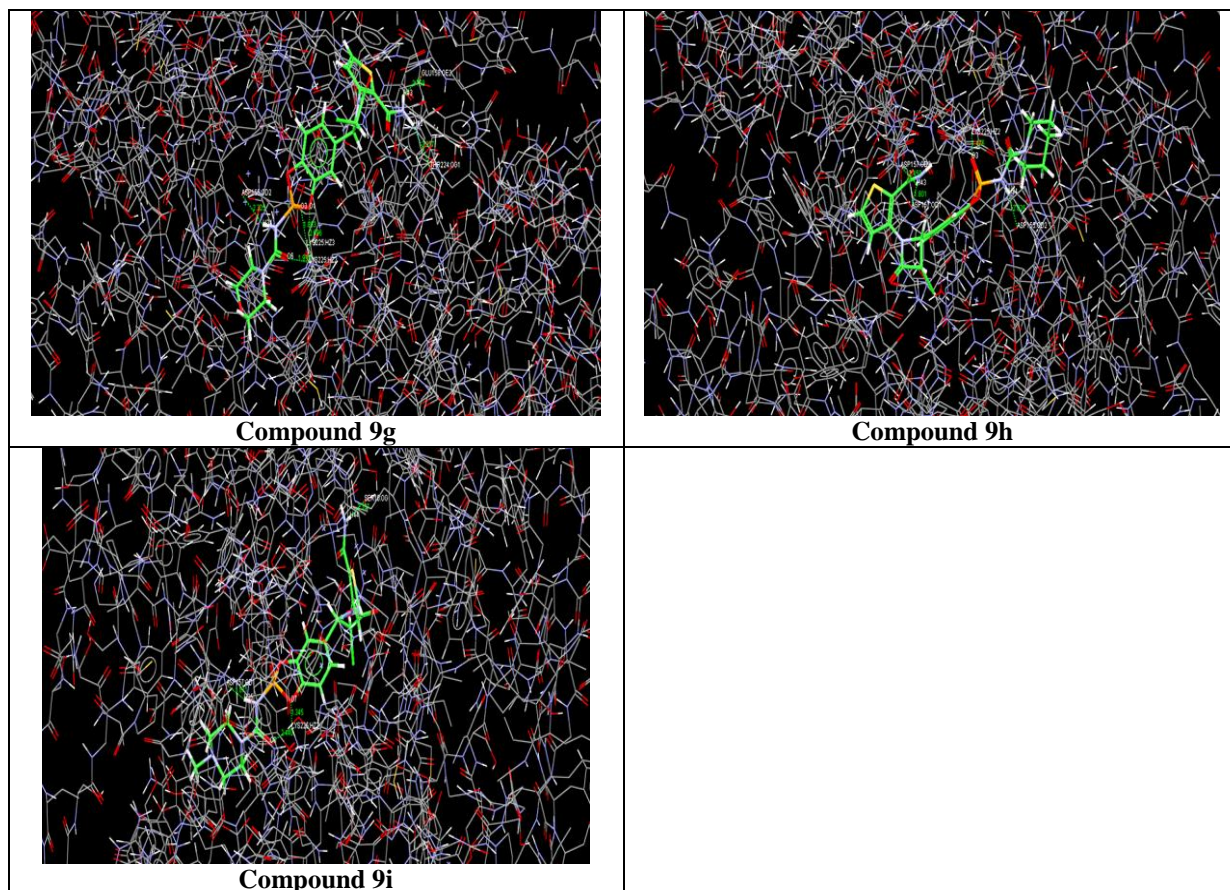


Fig. 3: Comparative Gold Score Fitness values for Compounds (9a-i)

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 (Å ^o)	Fitness
			Protein	Atoms		
9a	H	5	LYS225:HZ2	O14	1.555	46.91
			LYS225:HZ3	O6	2.372	
			LYS225:HZ1	O6	2.392	
			ASP155:OD2	H35	1.573	
			ASP155:OD2	H36	2.033	
9b	CH ₃	4	ASP155:OD2	H36	1.503	41.83
			ASP155:OD2	H37	2.121	
			LYS225:HZ2	O8	2.689	
			LYS225:HZ3	O14	1.588	
9c	OCH ₃	5	ASP157:OD1	H38	1.566	42.51
			ASP157:OD1	H37	2.228	
			LYS225:HZ3	O3	2.025	
			LYS225:HZ3	O14	2.542	
			LYS225:HZ2	O14	2.456	
9d	Cl	4	ASP155:OD2	H36	1.460	46.94
			ASP155:OD2	H37	2.617	
			LYS225:HZ1	O6	2.091	
			LYS225:HZ2	O14	1.434	
9e	CF ₃	5	ASP155:OD2	H46	1.652	44.14
			ASP155:OD2	H39	1.569	
			LYS225:HZ3	O11	1.678	
			LYS225:HZ1	O8	2.238	
			GLU158:O52	H53	3.382	
9f	NO ₂	6	ASP153:OD1	H38	2.081	42.51
			ASP153:OD1	H39	2.248	
			LYS225:HZ1	O1	2.463	
			LYS225:HZ2	O34	1.640	
			LYS225:HZ3	O3	2.397	
			LYS225:HZ3	O1	2.241	
9g	O	6	ASP155:OD2	H36	2.325	40.17
			LYS225:HZ2	O6	1.995	
			LYS225:HZ3	O3	1.863	
			LYS225:HZ2	O1	2.896	
			GLU158:OEZ	H43	2.159	
			THR224:OG1	H42	2.977	
9h	CH ₂	4	ASP157:OD2	H43	2.450	37.76
			ASP161:OD1	H43	1.801	
			ASP135:OD2	H34	2.026	
			LYS225:HZ2	O3	1.476	
9i	N-CH ₃	4	SER16:OG	H44	2.307	44.08
			LYS226:HZ2	O7	1.345	
			LYS226:HZ2	O6	2.440	
			ASP157:OD1	H35	1.871	

4. CONCLUSION

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

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India for providing facilities. The authors are also thankful to Central Drug Research Institute, Lucknow, India for supporting to do analytical data.

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