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T Sailaja Rani
Department of Chemistry, SVGM Govt.Degree College, Kalyandurg, Anantapur District, Andhra Pradesh, India

R Adinarayana Reddy
Department of Chemistry, Govt. College for Teacher Education, Kurnool, Andhra Pradesh, India

Corresponding Author:
T Sailaja Rani
Department of Chemistry, SVGM Govt. Degree College, Kalyandurg, Anantapur District, Andhra Pradesh, India

Synthesis, characterization, antimicrobial evaluation and docking studies of 3-(5-(2-oxido-2-(4-substituted phenyl)-ureido-benzo[*d*][1,3,2]dioxaphosphol)-5-yl-1*h*-tetrazol-1-yl) thiophene-2-carboxamides and carbamoyl thiophene-1*h*-tetrazolyl-dioxaphosphol-(morpholine/piperidine/4-methyl piperizine)-carboxamides

T Sailaja Rani and R Adinarayana Reddy

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Abstract
A series of compounds, 3-(5-(2-oxido-2-(4-substitutedphenyl)-ureido-benzo[d][1,3,2]dioxaphosphol)-5-yl-1H-tetrazol-1-yl)thiophene-2-carboxamides (9a-f), were synthesized through the condensation of 3-(5-(3,4-dihydroxyphenyl)-1H-tetrazol-1-yl)thiophene-2-carboxamide (7) with 4-substituted phenyl-carbamido-phosphoric acid dichlorides (8a-f). Similarly, carbamoyl thiophene-1H-tetrazolyl-dioxaphosphol morpholine/piperidine/4-methylpiperazine-carboxamides (9g-i) were obtained by condensing compound (7) with morpholinyl-, piperidinyl-, or N-methylpiperazinyl-carbamido-phosphoric acid dichlorides (8g-i).
Hydrolysis of 3-(5-(3,4-dimethoxyphenyl)-1H-tetrazol-1-yl) thiophene-2-carboxamide (6) produced the key intermediate (7). Compound (6) was synthesized via the reaction of 3-((3, 4-dimethoxybenzylidene) amino) thiophene-2-carboxamide (5) with sodium azide in tetrahydrofuran. Intermediate (5), in turn, was prepared by condensing 3-aminothiophene-2-carboxamide (3) with 3, 4-dimethoxybenzaldehyde (4) in the presence of a few drops of acetic acid. The starting compound (3) was obtained from the condensation of 2-cyanoacetamide (1) and 1,4-dithiane-2,5-diol (2) using trace amounts of triethylamine in ethanol.
The structures of the synthesized compounds (9a-i) were confirmed through IR, ^1H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis. The synthesized molecules were further subjected to biological evaluation and molecular docking studies, and the findings are presented in this article.

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

Introduction
Organo-phosphorus heterocyclic urea derivatives are widely recognized for their diverse pharmacological properties, including anti-infective [1], anti-tumor, anti-cancer, and antibacterial activities. Likewise, carboxamide derivatives containing heterocyclic moieties are known to inhibit the mammalian 5-lipoxygenase enzyme, highlighting their potential in the treatment of asthma, allergic disorders, and cardiovascular diseases [2].
Tetrazoles and their derivatives exhibit a broad spectrum of biological activities such as antifungal [3, 4], antinociceptive [5, 6], anticonvulsant [7], antidiabetic [8], cyclooxygenase inhibitory [9], hypoglycemic [10], antibacterial [11], and anti-inflammatory [12] effects. In addition, tetrazoles serve as effective catalysts in phosphonate synthesis. Considerable attention has also been directed toward dioxaphosphino ureas/carboxamides and their derivatives [13] within the realm of organophosphorus heterocyclic chemistry because of their significant biological applications [14, 15]. Motivated by these findings, we aimed to design and synthesize novel tetrazole-urea derivatives of organophosphorus compounds incorporating a thiophene moiety. In this study, we report the synthesis of 3-(5-(2-oxido-2

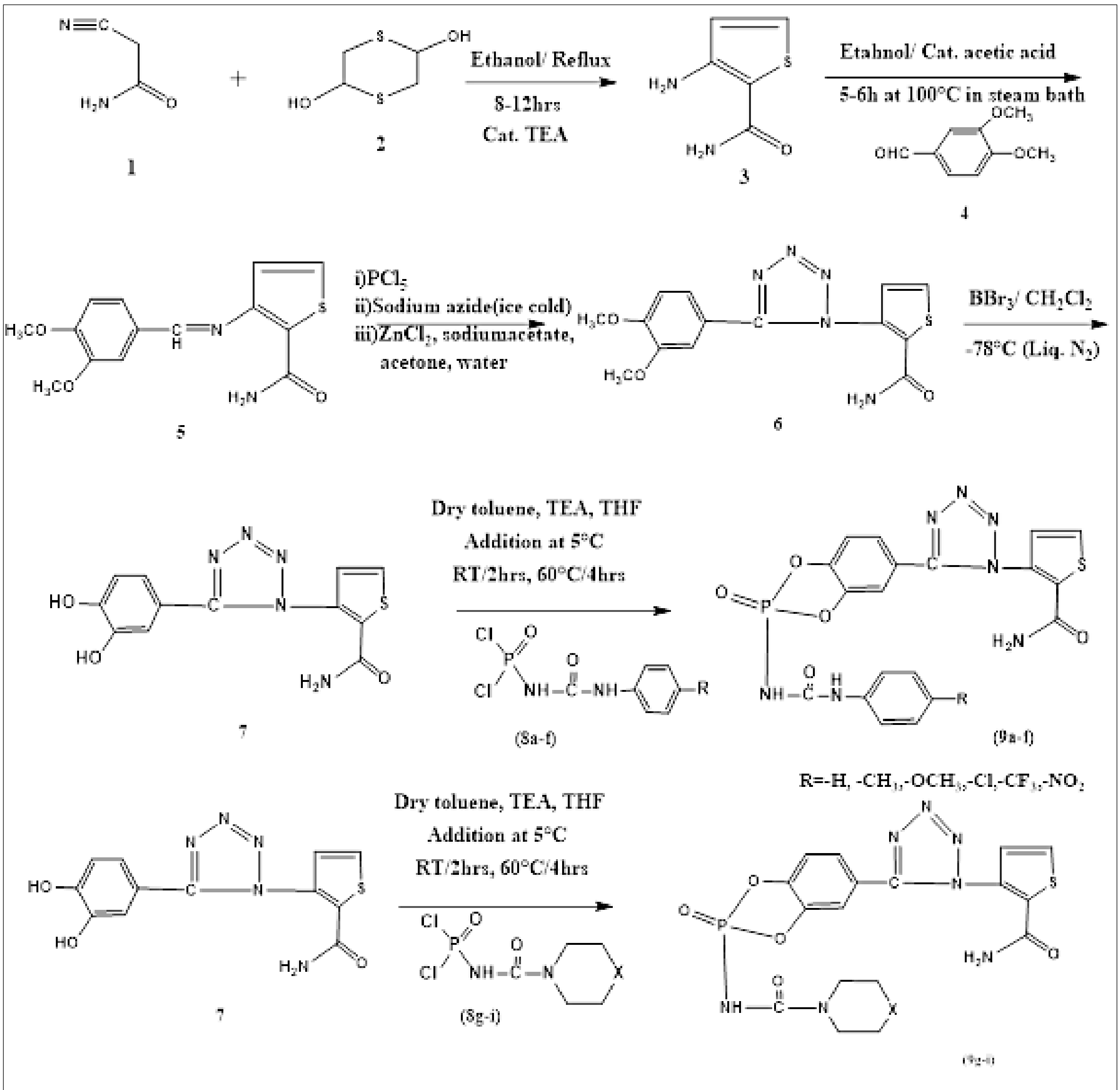
-(4-substituted phenyl)-ureido-benzo[d][1,3,2] dioxaphosphol)-5-yl-1H-tetrazol-1-yl) thiophene-2-carboxamides (9a-f) and carbamoyl thiophene-1H-tetrazolyl-dioxaphosphol morpholine/piperidine/4-methylpiperazine-carboxamides (9g-i). The chemical structures of these compounds were confirmed through elemental analysis, IR, NMR, and mass spectroscopic techniques. All synthesized compounds were evaluated for antimicrobial activity, with several derivatives demonstrating promising results.

Materials and Methods

All reagents and chemicals employed in this study were procured from Sigma-Aldrich, Inc. (USA) and used without further purification. Thin-layer chromatography (TLC) was performed on aluminum sheets coated with silica gel $60F_{254}$ (E. Merck, Germany), using iodine vapor as the visualizing agent. Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are reported uncorrected.

Purification of the synthesized compounds was achieved by column chromatography on silica gel, using various solvent systems as eluents. Infrared (IR) spectra were recorded on a Perkin-Elmer 1000 unit using KBr pellets. ^1H and ^{13}C NMR spectra were obtained in DMSO-d_6 on a Varian XL-300 spectrometer operating at 400 MHz and 75 MHz, respectively, while the ^{31}P NMR spectrum was recorded on a Varian XL spectrometer at 161.89 MHz. Tetramethylsilane (TMS) and 85% H_3PO_4 served as internal standards for $^1H/^{13}C$ NMR and ^{31}P NMR, respectively. Elemental analysis was carried out on a Carlo Erba 1108 Elemental Analyzer at the Central Drug Research Institute, Lucknow, India.

Compounds (9a-i) were synthesized in 5 steps as presented in the Scheme-I



Scheme -1: *Proposed synthetic scheme for the preparation of (9a-i)*

Synthesis of 3-aminothiophene-2-carboxamide (3) [16, 17]

A mixture of 2-cyanoacetamide (1, 0.02 mol) and 2, 5-dihydroxy-1, 4-dithiane (2, 0.025 mol) in ethanol (50 mL) was refluxed for 8 hours in the presence of a catalytic amount of triethylamine. The reaction progress was monitored by TLC (alumina plates, n-hexane:ethyl acetate = 7:3). After completion, the solvent was evaporated under reduced pressure, and the residue was cooled to room

temperature. Isopropyl alcohol was added, and the mixture was kept at room temperature for 1 hour. The resulting solid was filtered, washed, and dried under suction, followed by recrystallization from 2-propanol.
Yield: 0.015 mol (75%); M.P.: 122-124 °C.
IR (KBr, cm^{-1}): 3400, 3450 (amide N-H stretch), 3330, 3345 (amine N-H stretch), 3035 (aromatic C-H), 1674 (amide I), 1491, 677 (thiophene ring).
^{1}H NMR (DMSO-d_6, δ ppm): 5.20 (bs, 2H, -NH_2 attached to thiophene), 7.80 (s, 2H, -NH_2 of amide), 6.98-7.20 (m, 2H, thiophene protons).

Synthesis of 3-((3, 4-Dimethoxybenzylidene) amino) thiophene-2-carboxamide (5)
Equimolar amounts of 3, 4-dimethoxybenzaldehyde (4, 0.02 mol) and 3-aminothiophene-2-carboxamide (3, 0.02 mol) were dissolved in absolute ethanol (50 mL) with three drops of acetic acid, and the mixture was heated on a steam bath at 100 °C for 5 hours. Reaction completion was checked by TLC (alumina plates). The mixture was kept at room temperature for 24 hours, and the product was recrystallized from warm absolute ethanol.
Yield: 0.015 mol (75%); M.P.: 160-162 °C.
IR (KBr, cm^{-1}): 3400, 3450 (amide N-H), 3052 (aromatic C-H), 1674 (amide I), 1620 (C= N of azomethine), 1491, 677 (thiophene), 1050 (C-O-C stretch of aromatic ether).
^{1}H NMR (DMSO-d_6, δ ppm): 3.80 (s, 6H, 2 × -OCH_3), 7.80 (s, 2H, amide -NH_2), 6.98-7.30 (m, 5H, aromatic + thiophene H), 8.30 (s, 1H, CH = N).

Synthesis of 3-(5-(3, 4-Dimethoxyphenyl)-1H-tetrazol-1-yl) thiophene-2-carboxamide (6)
Compound (5) was treated with PCl_5, followed by an ice-cold solution of sodium azide in THF, in the presence of $ZnCl_2$ and excess sodium acetate (aqueous-acetone medium, stirred). Reaction progress was monitored by TLC (n-hexane:ethyl acetate = 7:3).
Yield: 0.015 mol (75%); M.P.: 123-125 °C.
IR (KBr, cm^{-1}): 3400, 3450 (amide N-H), 3052 (aromatic C-H), 1674 (amide I), 1483, 696 (thiophene), 1050 (C-O-C of aromatic ether), 1453, 1415, 1299, 1129, 1074 (tetrazole).
^{1}H NMR (DMSO-d_6, δ ppm): 3.80 (s, 6H, 2 × -OCH_3), 7.80 (s, 2H, amide -NH_2), 7.01-7.30 (m, 5H, aromatic + thiophene H).

Synthesis of 3-(5-(3, 4-Dihydroxyphenyl)-1H-tetrazol-1-yl) thiophene-2-carboxamide (7)
A solution of (6, 0.02 mol) in CH_2Cl_2 (30 mL) was cooled under liquid N_2, and boron tribromide (2.4 mL, 0.025 mol) was added at -78 °C. The mixture was slowly warmed to room temperature and stirred for 16 hours. TLC monitoring confirmed reaction completion. The reaction was quenched with cold methanol and ice water, followed by pH adjustment to 7-8 using saturated $NaHCO_3$. The product was extracted with ethyl acetate (3 × 25 mL), and the combined organic layers were dried over anhydrous Na_2SO_4, concentrated, and purified by column chromatography (petroleum ether:ethyl acetate = 4:1).
Yield: 0.015 mol (75%); M.P.: 163-165 °C.
IR (KBr, cm^{-1}): 3350 (O-H stretch, intramolecular H-bond), 3400, 3450 (amide N-H), 3052 (aromatic C-H), 1674 (amide I), 1483, 696 (thiophene), 1453, 1415, 1299, 1129, 1074 (tetrazole).
^{1}H NMR (DMSO-d_6, δ ppm): 7.80 (s, 2H, amide -NH_2), 7.01-7.30 (m, 5H, aromatic + thiophene H), 5.60 (s, 2H, 2 × -OH).

Synthesis of 4-Substituted Phenyl/Morpholinyl/ Piperidinyl/N-Methylpiperazinyl-carbamido-phosphoric Acid Dichlorides (8a-i)
The compounds (8a-i) were prepared according to literature procedures [22, 23].
General Procedure for the Synthesis of Target Compounds (9a-i).
To a stirred solution of compound (7, 0.02 mol) and triethylamine (0.04 mol) in dry toluene (30 mL) and tetrahydrofuran (10 mL), a solution of (phenylcarbamoyl) phosphoramidic dichloride (8a, 0.02 mol) in dry toluene (25 mL) was added dropwise over 20 minutes at 5 °C. After addition, the reaction mixture was stirred at room temperature for 2 hours, then heated to 50-60 °C for 4 hours. Progress was monitored by TLC. The resulting triethylamine hydrochloride was filtered off, and the filtrate was concentrated under reduced pressure. The residue was washed with water and recrystallized from aqueous 2-propanol to afford 3-(5-(2-oxido-2-(3-phenylureido) benzo[d][1, 3, 2] dioxaphosphol-5-yl)-1H-tetrazol-1-yl)thiophene-2-carboxamide (9a).
Yield: 0.014 mol (70%); M.P.: 110-112 °C.
The same procedure was followed for the synthesis of compounds (9b-f) using the corresponding p-tolyl-, 4-methoxyphenyl-, 4-chlorophenyl-, 4-(trifluoromethyl) phenyl-, and 4-nitrophenyl-carbamoyl phosphoramidic dichlorides (8b-f). Likewise, reactions of (7) with (morpholine-4-carbamoyl) phosphoramidic dichloride (8g), (piperidine-1-carbamoyl) phosphoramidic dichloride (8h), and (4-methylpiperazine-1-carbamoyl) phosphoramidic dichloride (8i) afforded compounds (9g-i), respectively.
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Results and Discussion

Spectral, Physical and Analytical data for the compounds (9a-i)
9a: Yield: 70%; m.p.: 110-112 °C.
Anal. Calcd for $C_{19}H_{14}N_7O_5PS$ (%): C, 46.58; H, 2.43; N, 19.28; P, 5.93; S, 6.15.
IR (KBr, ν, cm^{-1}): 3450, 3400 (N-H, amide), 3317 (N-H, P-NH), 3030 (C-H, Ar-H of benzene and thiophene rings), 1670 (C=O, amide), 1656 (C=O, ureido), 1483, 696 (thiophene), 1415, 1285, 1138, 1105 (tetrazole ring), 1250 (P=O), 954 (P-O).
^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.95 (s, 1H, CO-NH-PO), 7.00-8.15 (m, 10H, C_6H_3, C_6H_5 and C_4H_2S), 7.04 (s, 2H, $CONH_2$), 8.85 (s, 1H, CO-NH-Ar).
^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 144.3, 135.8, 129.0, 156.5, 163.5, 124.6, 114.3, 145.7, 145.2, 117.8, 123.3, 152.0, 139.4, 121.6, 128.9, 128.0, 128.9, 121.6, 162.3 (corresponding to C_1-C_{19}, 17 non-equivalent carbons).
^{31}P NMR (161.89 MHz, DMSO-d_6, δ ppm): -8.0.
MS (m/z): 483 [M^+].
NMR (δ, ppm): -8.0, Mass (m/z): 483 M^{+}.
9b: Yield: 70%; m.p.: 115-117 °C.
Anal. Calcd for $C_{20}H_{16}N_7O_5PS$ (%): C, 47.78; H, 2.80; N, 19.22; P, 5.76; S, 5.97.
IR (KBr, ν, cm^{-1}): 3410, 3390 (N-H, amide), 3320 (N-H, P-NH), 3025 (C-H, aromatic), 1665 (C=O, amide), 1660 (C=O, ureido), 1483, 696 (thiophene), 1410, 1280, 1133, 1100 (tetrazole ring), 1245 (P=O), 950 (P-O). .

^{1}H-NMR (DMSO-d_6), δ ppm (J, Hz):2.27(s, 3H, CH_3), 5.95(s, 1H, CO-NH-PO), 7.04(s, 2H, $CONH_2$), 7.05-8.15 (m, 9H, C_6H_4, C_6H_3 & C_4H_2S) and 8.85(s, 1H, CO-NH-Ar). P^{31}-NMR (δ, ppm): -8.5.

9c: Yield: 70%; m.p.: 80-82 °C.

Anal. Calcd for $C_{20}H_{16}N_7O_6PS$ (%): C, 46.28; H, 2.57; N, 18.50; P, 5.52; S, 5.77.

IR (KBr, ν, cm^{-1}): 3415, 3395 (N-H, amide), 3325 (N-H, P-NH), 3040 (C-H, aromatic), 1670 (C=O, amide), 1665 (C=O, ureido), 1483, 696 (thiophene), 1415, 1285, 1138, 1105 (tetrazole ring), 1254 (P=O), 958 (P-O).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.75 (s, 3H, OCH_3), 5.95 (s, 1H, CO-NH-PO), 6.87-8.15 (m, 9H, C_6H_4, C_6H_3, and C_4H_2S), 7.04 (s, 2H, $CONH_2$), 8.85 (s, 1H, CO-NH-Ar).

^{31}P NMR (161.89 MHz, DMSO-d_6, δ ppm): -7.6.

9d: Yield: 70%; m.p.: 122-124 °C.

Anal. Calcd for $C_{19}H_{13}ClN_7O_5PS$ (%): C, 43.52; H, 2.00; Cl, 6.38; N, 18.55; P, 5.50; S, 5.70.

IR (KBr, ν, cm^{-1}): 3420, 3405 (N-H, amide), 3330 (N-H, P-NH), 3035 (C-H, aromatic), 1675 (C=O, amide), 1670 (C=O, ureido), 1483, 696 (thiophene), 1417, 1290, 1142, 1110 (tetrazole ring), 1256 (P=O), 956 (P-O).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.95 (s, 1H, CO-NH-PO), 7.04 (s, 2H, $CONH_2$), 7.20-8.15 (m, 9H, C_6H_4, C_6H_3, and C_4H_2S), 8.85 (s, 1H, CO-NH-Ar).

^{31}P NMR (161.89 MHz, DMSO-d_6, δ ppm): -8.0.

9e: Yield: 70%. m.p:128-130°C. Anal. Data Found for $C_{20}H_{13}F_3N_7O_5PS$(%): C 43.05, H 1.90, F9.48, N 17.29, P 5.17and S 5.34; IR(KBr pellet), $\bar{\nu}$, cm^{-1}: 3420 & 3447 (N-H str, amide), 3335(N-H str, P-NH), 3039(C-H str, Ar), 1680(C=O str, amide), 1675(C=O str, ureido), 1483, 696(thiophene), 1420, 1293, 1146 & 1113(tetrazole ring), 1260(P=O str.) and 964(P-O str.);^{1}H-NMR (DMSO-d_6), δ, ppm (J, Hz): 5.95(s, 1H, CO-NH-PO), 7.04(s, 2H, $CONH_2$), 7.20-8.15(m, 9H, C_6H_4, C_6H_3 & C_4H_2S) and 8.85(s, 1H, CO-NH-Ar). P^{31}-NMR (δ, ppm): -8.7.

9f: Yield: 70%; m.p.: 147-149 °C.

Anal. Calcd for $C_{19}H_{13}N_8O_7PS$ (%): C, 42.68; H, 1.97; N, 20.73; P, 5.38; S, 5.53.

IR (KBr, ν, cm^{-1}): 3450, 3430 (N-H, amide), 3340 (N-H, P-NH), 3040 (C-H, aromatic), 1690 (C=O, amide), 1680 (C=O, ureido), 1483, 696 (thiophene), 1425, 1300, 1153, 1120 (tetrazole ring), 1270 (P=O), 974 (P-O).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.95 (s, 1H, CO-NH-PO), 7.04 (s, 2H, $CONH_2$), 7.20-8.10 (m, 9H, C_6H_4, C_6H_3, and C_4H_2S), 8.85 (s, 1H, CO-NH-Ar).

^{31}P NMR (161.89 MHz, DMSO-d_6, δ ppm): -9.3.

9g: Yield: 70%; m.p.: 88-90 °C.

Anal. Calcd for $C_{17}H_{16}N_7O_6PS$ (%): C, 42.24; H, 2.88; N, 20.02; P, 5.98; S, 6.23.

IR (KBr, ν, cm^{-1}): 3425, 3410 (N-H, amide), 3328 (N-H, P-NH), 3045 (C-H, aromatic), 1675 (C=O, amide), 1662 (C=O, ureido), 1483, 696 (thiophene), 1420, 1282, 1136, 1102 (tetrazole ring), 1260 (P=O), 964 (P-O).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.25-3.50 (m, 8H, morpholine ring), 5.95 (s, 1H, CO-NH-PO), 7.04 (s, 2H, $CONH_2$), 7.20-8.15 (m, 5H, C_6H_3 and C_4H_2S).

^{31}P NMR (161.89 MHz, DMSO-d_6, δ ppm): -7.6.

9h: Yield: 70%; m.p.: 102-104 °C.

Anal. Calcd for $C_{18}H_{18}N_7O_5PS$ (%): C, 44.96; H, 3.32; N, 20.12; P, 6.00; S, 6.22.

IR (KBr, ν, cm^{-1}): 3425, 3410 (N-H, amide), 3331 (N-H, P-NH), 3050 (C-H, aromatic), 1665 (C=O, amide), 1660 (C=O, ureido), 1483, 696 (thiophene), 1417, 1280, 1133, 1100 (tetrazole ring), 1250 (P=O), 954 (P-O).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.54 (q, 2H, piperidine), 1.67-3.77 (m, 8H, piperidine), 5.95 (s, 1H, CO-NH-PO), 7.04 (s, 2H, $CONH_2$), 7.20-8.15 (m, 5H, C_6H_3 and C_4H_2S).

^{31}P NMR (161.89 MHz, DMSO-d_6, δ ppm): -7.9.

9i: Yield: 70%; m.p.: 113-115 °C.

Anal. Calcd for $C_{18}H_{19}N_8O_5PS$ (%): C, 43.50; H, 3.42; N, 22.34; P, 5.84; S, 6.01.

IR (KBr, ν, cm^{-1}): 3420, 3405 (N-H, amide), 3330 (N-H, P-NH), 3055 (C-H, aromatic), 1670 (C=O, amide), 1665 (C=O, ureido), 1483, 696 (thiophene), 1420, 1285, 1138, 1105 (tetrazole ring), 1256 (P=O), 956 (P-O).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.13 (s, 3H, N-CH_3), 2.22-3.34 (m, 8H, piperazine), 5.95 (s, 1H, CO-NH-PO), 7.04 (s, 2H, $CONH_2$), 7.20-8.15 (m, 5H, C_6H_3 and C_4H_2S).

^{31}P NMR (161.89 MHz, DMSO-d_6, δ ppm): -7.8.

Biological activity: The physical and biological properties of chemical compounds play a crucial role in determining their antimicrobial potential [25, 26]. It is well recognized that a correlation exists between the chemical structure of a compound and its biological activity [27]. Heterocyclic organic molecules incorporating phosphorus, oxygen, nitrogen, or sulfur atoms within their ring systems often exhibit enhanced biological activity, as these heteroatoms facilitate diverse molecular interactions [28, 29].

In light of this, the newly synthesized organophosphorus heterocyclic compounds were evaluated for their antimicrobial efficacy. The antimicrobial screening was carried out using the disc diffusion method, following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [30]. The test compounds were dissolved in DMF at a concentration of 250 µg/mL, with amoxicillin serving as the reference standard for antibacterial activity and ketoconazole as the standard for antifungal activity.

Antibacterial activity: The antibacterial activity [31] of the synthesized compounds — namely, 3-(5-(2-oxido-2-(3-phenylureido)/(3-(p-tolyl)ureido)/(3-(4-methoxyphenyl)ureido)/(4-chlorophenyl)ureido)/(4-(trifluoromethyl)phenyl)ureido)/(4-nitrophenyl)ureido)-2-oxidobenzo[d][1, 3, 2]dioxaphosphol-5-yl)-1H-tetrazol-1-yl)thiophene-2-carboxamides (9a-f) and N-(5-(1-(2-carbamoylthiophen-3-yl)-1H-tetrazol-5-yl)-2-oxidobenzo[d][1, 3, 2]dioxaphosphol-2-yl)morpholine-4-carboxamide/piperidine-1-carboxamide/4-methylpiperazine-1-carboxamide (9g-i) — was evaluated against both Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*).

In the current investigation, most of the synthesized compounds demonstrated moderate to good antibacterial activity against both bacterial strains. Within the first series (9a-f), compounds containing nitro (9f), trifluoromethyl (9e), and chloro (9d) substituents exhibited notably higher antibacterial activity compared to the other derivatives. Among the second series (9g-i), the piperidine-substituted compound (9h) displayed superior antibacterial potency relative to its morpholine (9g) and 4-methylpiperazine (9i) analogues.

The antibacterial results for compounds 9a-f are presented in Table 1, while those for 9g-i are listed in Table 2. The overall antibacterial activity profiles of all synthesized compounds are also depicted graphically in Figure 1.
The order of anti-bacterial activity of the first series compounds (9a-f) found to be:
9f>9e>9d>9a>9b>9c
The order of anti-bacterial activity found in the second series of compounds (9g-i):
9h>9i>9g

Table 1: Antibacterial activity (Diameter of 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	11	09	10	09
2	9b	09	08	08	08
3	9c	08	06	07	07
4	9d	13	11	12	11
5	9e	16	10	14	13
6	9f	18	13	15	14
Amoxicillin		21	27	24	22

Table 2: Antibacterial activity (Diameter of 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	13	10	11	10
2	9h	17	15	14	13
3	9i	14	12	13	12
Amoxicillin		21	27	24	22

Antifungal activity: The antifungal activity of the synthesized compounds — 3-(5-(2-oxido-2-(3-phenylureido)/(3-(p-tolyl)ureido)/(3-(4-methoxyphenyl)ureido)/(4-chlorophenyl)ureido)/(4-(trifluoromethyl)phenyl)ureido)/(4-nitrophenyl)ureido)-2-oxidobenzo[d][1, 3, 2]dioxaphosphol-5-yl)-1H-tetrazol-1-yl)thiophene-2-carboxamides (9a-f) and N-(5-(1-(2-carbamoylthiophen-3-yl)-1H-tetrazol-5-yl)-2-oxidobenzo[d][1, 3, 2]dioxaphosphol-2-yl)morpholine-4-carboxamide/piperidine-1-carboxamide/4-methylpiperazine-1-carboxamide (9g-i) — was evaluated against Aspergillus niger and Candida albicans [32].
Among the first series (9a-f), the derivatives bearing nitro (9f), trifluoromethyl (9e), and chloro (9d) substituents exhibited stronger antifungal activity compared to other analogues. Similarly, within the second series (9g-i), the piperidine-substituted compound (9h) demonstrated the highest antifungal potency among the tested derivatives.
The antifungal screening data for compounds 9a-f and 9g-i are presented in Tables 3 and 4, respectively, while the comparative antifungal activities of all synthesized compounds (9a-i) are graphically illustrated in Figure 2.
The order of anti-fungal activity found in the first series compounds (9a-f):
9f>9e>9d>9a>9b>9c
The order of anti-fungal activity found in the second series of compounds (9g-i):
9h>9i>9g

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	08
2	9b	09	07
3	9c	07	06
4	9d	14	12
5	9e	16	14
6	9f	18	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	13	11
2	9h	17	15
3	9i	14	12
Ketoconazole		22	25

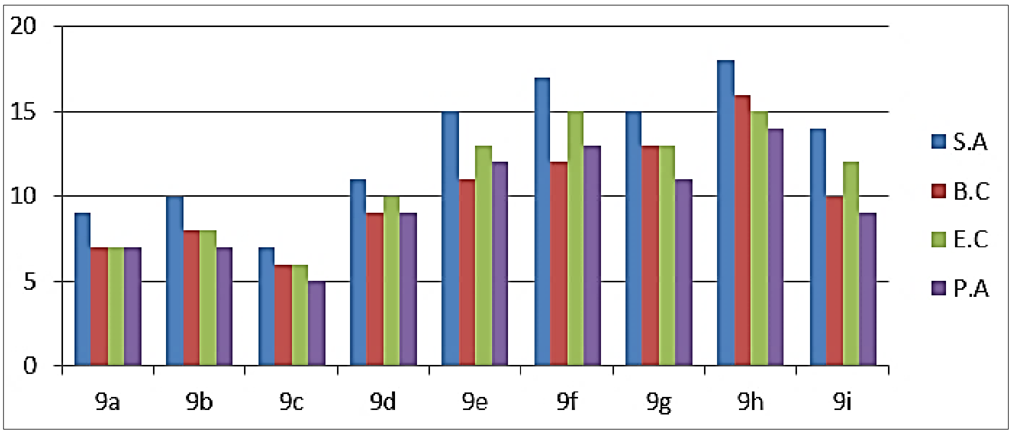


Fig 1: Graphical representation of antibacterial activity of synthesized compounds.

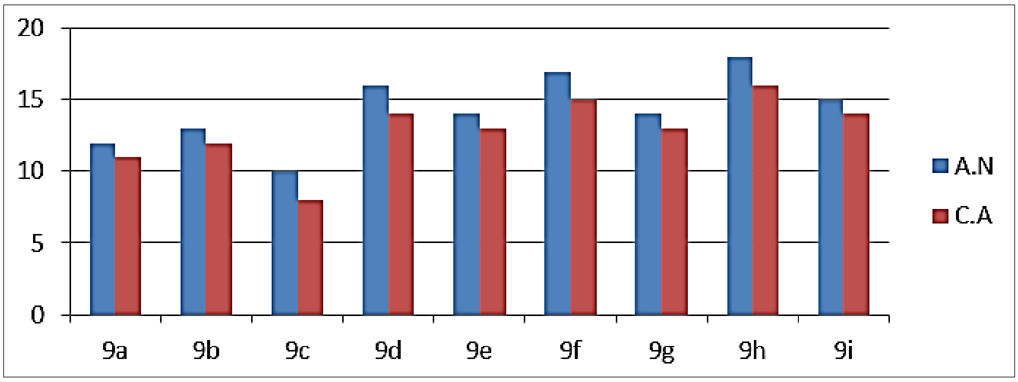


Fig 2: Comparative antifungal activity of synthesized compounds (9a–i).

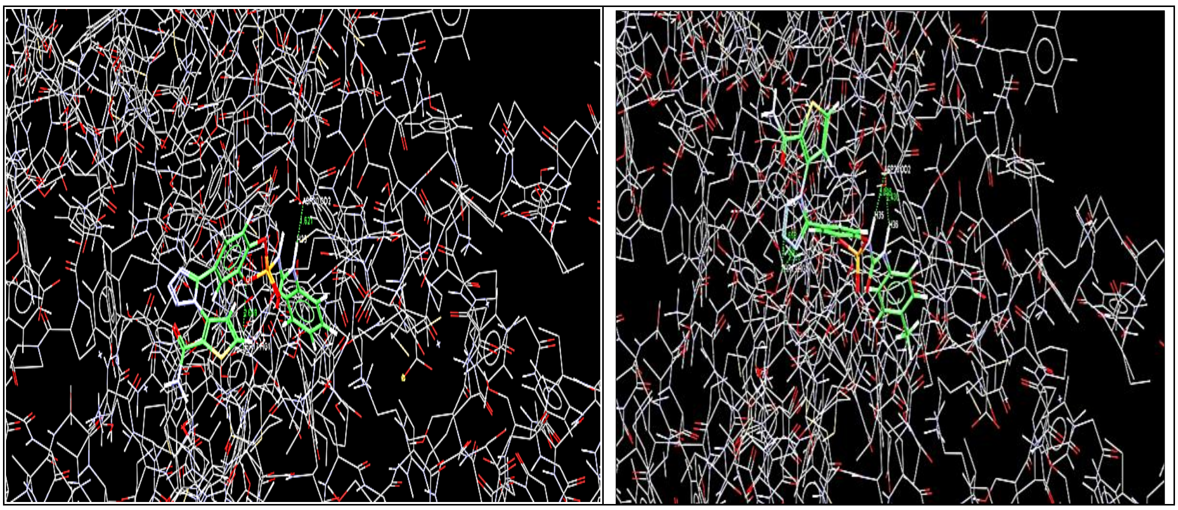
Docking Studies of the compounds (9a-i): The docking results revealed that all ligands exhibited specific interactions between the oxygen atoms of the ligands and the amino acid residues within the active site of the receptor. The findings of the docking studies are summarized in Tables 5-6 and illustrated in Figure 3. The docked conformations showed that the ligands formed hydrogen-bonding interactions with several key residues, including Aspartic acid (Asp-90, Asp-20), Threonine (Thr-13, Thr-17), Lysine (Lys-275), and Glutamine (Gln-24). These residues were consistently involved in common hydrogen bonds across all docked complexes.

The van der Waals interaction scores for the ligand-protein complexes followed the order:

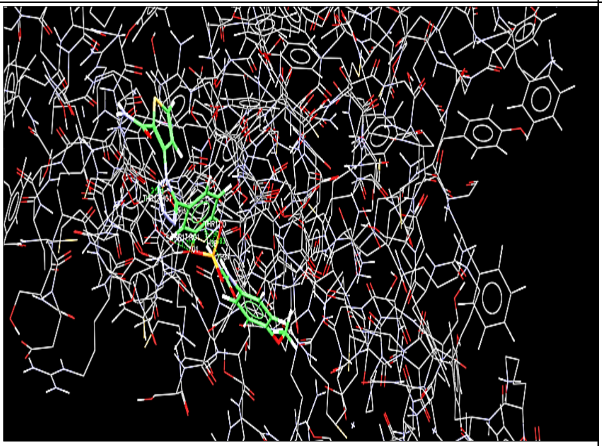
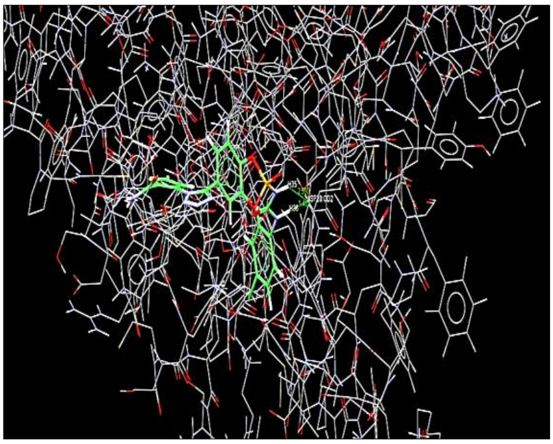
Similarly, the GOLD fitness scores indicated that compound 9h exhibited the strongest binding affinity, while compound 9f displayed the weakest interaction with the Histamine H_1 receptor. Comparative GOLD fitness scores for all ligands (9a-i) are presented graphically in Figure 4.

All ligands demonstrated moderate to good binding affinities with the receptor and showed minimal intramolecular strain, indicating stable docked conformations. The results suggest that electronic, hydrogen-bonding, and steric interactions play crucial roles in determining ligand binding efficiency. Furthermore, the electronic nature of substituents significantly influences both docking performance and observed antimicrobial activity.

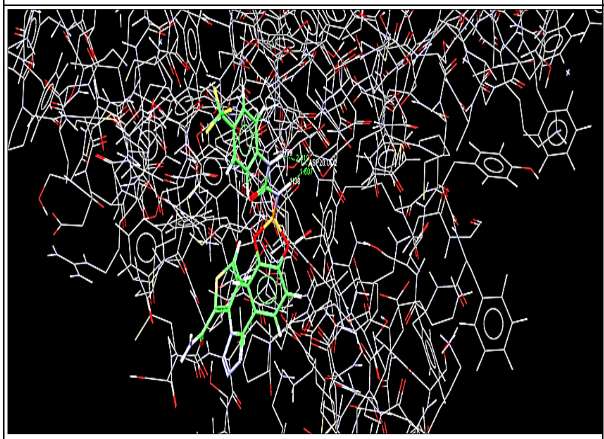
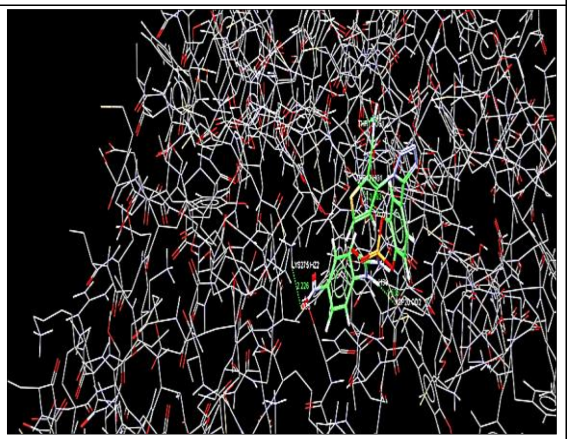
9h > 9d > 9a > 9b > 9g > 9c > 9e > 9i > 9f.



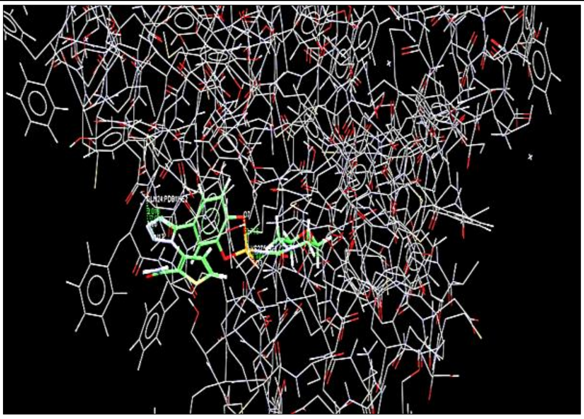
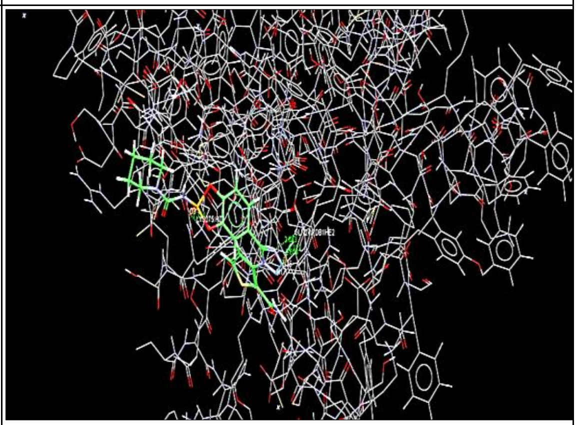
9a	9b

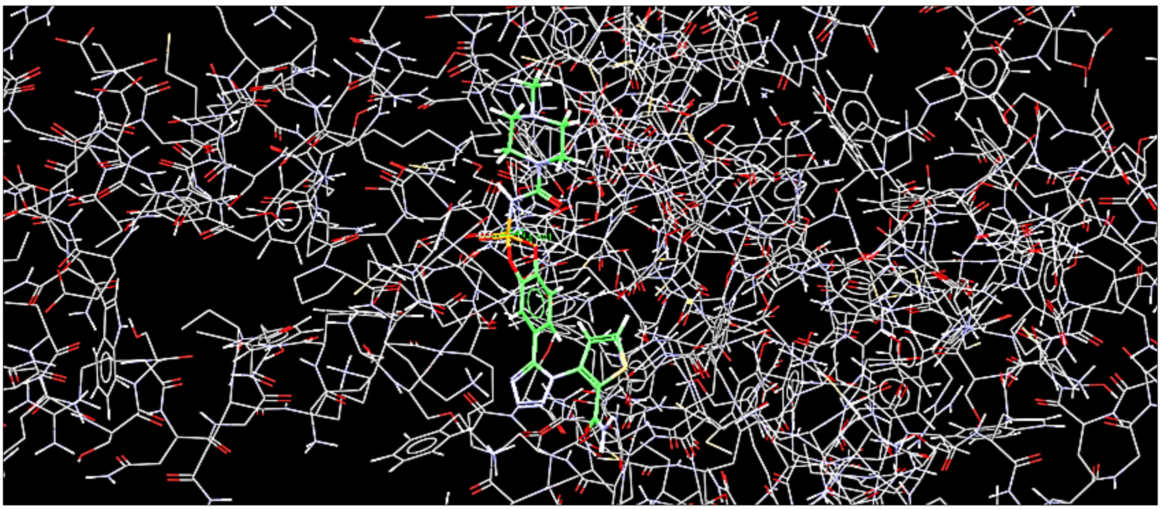
9c	9d

9e	9f

9g	9h



9i

Fig 3: Docking activity of compounds (9a-i) with H_1 Receptor protein

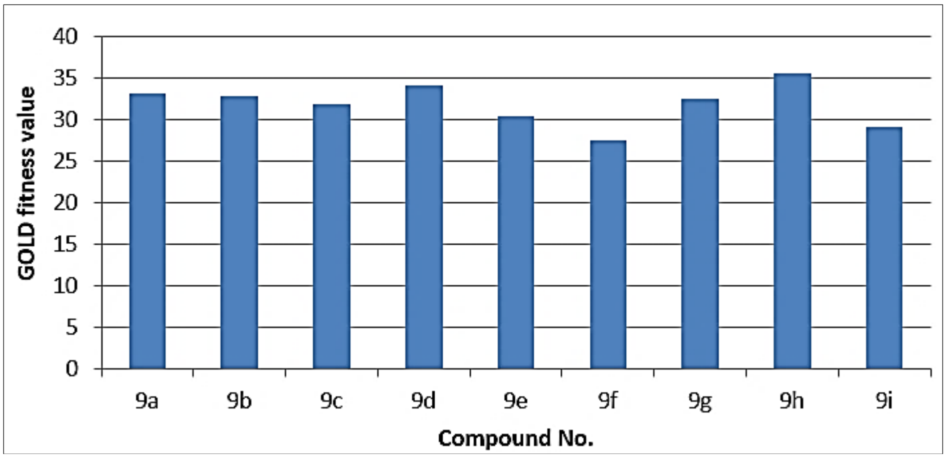


Fig 4: Comparative Gold Score Fitness values for Compounds (9a-i)

Table 5: Docking results of (9a-i) on H_1 Receptor Protein (Histamine Receptor) protein

Comp	R/X	Fitness	S(Hb_ext)	S(vdw_ext)	S(Hb_int)	S(vdw_int)
9a	H	33.08	5.75	28.84	0.00	-12.32
9b	CH_3	32.85	7.55	26.42	0.00	-11.02
9c	OCH_3	31.85	4.19	28.45	0.00	-11.46
9d	Cl	34.06	5.71	30.07	0.00	-13.00
9e	CF_3	30.35	6.41	25.07	0.00	-10.53
9f	NO_2	27.51	10.83	22.41	0.00	-14.14
9g	O	32.47	3.06	27.18	0.00	-7.97
9h	CH_2	35.64	5.47	27.88	0.00	-8.17
9i	N-CH_3	29.10	6.00	23.32	0.00	-8.97

Table 6: Hydrogen bonding interactions of Compounds (9a-i) with H_1 Receptor Protein (Histamine Receptor)

Comp No	R/X	No of 'H' bonds	Compounds		Bond Length (A°)	Fitness
			Protein	Atoms		
9a	H	2	ASP90:OD2 THR13:HG1	H25 O1	1.621 2.669	33.08
9b	CH_3	4	ASP20:OD2 ASP20:OD2 THR13:HG1 THR13:HG1	H35 H36 N24 O35	2.036 2.430 2.658 2.526	32.86
9c	OCH_3	4	THR17:O THR17:O THR13:HG1 THR13:HG1	H37 H38 O1 N24	1.683 1.790 1.305 2.576	31.85
9d	Cl	2	ASP20:OD2 ASP20:OD2	H35 H36	2.593 1.489	34.06
9e	CF_3	2	ASP20:OD2 ASP20:OD2	H38 H39	1.607 2.918	30.35
9f	NO_2	4	ASP20:OD2 LYS275:HZ2 THR17:HG1 THR13:OG1	H39 O35 O1 H48	1.635 2.226 2.682 2.453	27.50
9g	O	4	LYS275:HZ3 LYS275:HZ3 GLN24:PDB1HE2 GLN24:PDB1HE2	O3 O7 N26 N17	1.804 2.716 2.018 2.046	32.46
9h	CH_2	4	GLN24:PDB1HE2 GLN24:PDB1HE2 GLN24:PDB1HE2 LYS275:HZ3	N26 N17 O3	2.623 4.222 3.864 1.324	35.64
9i	N-CH_3	2	LYS275:HZ3 LYS275:HZ3	O3 O2	2.545 1.681	29.10

4. Conclusion

In present research work, few analogues of 3-(5-(2-oxido-2-(4-substituted phenyl)-ureido-benzo[*d*][1,3,2] dioxaphosphol)-5-yl-1*H*-tetrazol-1-yl)thiophene-2-carboxamides(9a-f) and Morpholine/Piperidine/4-methyl piperizine carboxamides (9g-i) were successfully prepared, characterized and screened for Biological activity and docking studies.

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