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# Synthesis, Characterization, Docking and Antimicrobial Evaluation of Novel Compounds of 2-Phenoxy-1,3,2-Benzodioxaphosphole-2-Oxide-Oxo Azetidin and Pyrazol-5-One-Mannich Bases

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Research Article

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## ABSTRACT

The synthetic route of new mannich bases of 4-(3-chloro-1-(2-oxido-2-(4-substituted phenoxy) [d] dioxaphosphole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(tri fluoromethyl)-1H-pyrazole-5(4H)-one(8a-f) was depicted in scheme:1. The mannich bases (8a-f) were prepared by condensation reaction between 4-(3-chloro-1-(3,4–dihydroxyphenyl)-4-oxaazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one(6) and 4-Substituted-PhenylPhosphorodichlori date (7a-f). The synthon was obtained by hydrolysis of 4-(3-chloro-1-(3,4-dimethoxyphenyl)-4-oxo azetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5-(4H)-one(5). The synthon was obtained by condensation reaction between 4-(((3,4-dimethoxyphenyl)imino)methyl)-1-(morpholinomethyl)-3-(trifluoro methyl)-1H-pyrazol-5-(4H)-one(4) and chloroacetyl chloride. The synthon was obtained by Mannic reaction between 4-(((3,4-dimethoxy phenyl)imino)methyl)-3-(trifluoromethyl)-1-1H-pyrazol-5-(4H)-one(3) with formaldehyde and morpholine. The synthon was obtained by condensation between 5-oxo-3-(trifluoromethyl)-4,5-dihydro-1H-pyrazole-4-carbaldehyde and 3-4-dimethoxy aniline.

The structures of newly synthesized compounds (8a-f) were established by IR, 1HNMR, 13C-NMR, 31P-NMR, Mass spectral studies and Elemental analysis.

## INTRODUCTION

Heterocyclic compounds represent an important class of biologically active molecules especially, those containing the pyrazolone nucleus have been shown to possess high biological activities [1-3] such as anticancer, antischenic effects, anti-inflammatory, antifungal, antipyretic, antitubercular, antihypertensive, antiviral [4] and antimicrobial. The derivatives of pyrazolones are an important class of antipyretic and analgesic compounds. Organophosphorus compounds have attracted the attention of researches because of their multifaceted applications in industrial, agricultural, biochemical, and medicinal areas. organophosphorus esters are being used as pesticides and insecticides. It is an established fact that compounds containing four-membered and five-membered heterocyclic ring systems exhibit potent biological effects like antimicrobial, anticonvulsant, anti-inflammatory, anti-cancer,antitubercular, antiviral activities. With reference to azetidine-2-ones, these are important class of structural moieties having four-membered heterocyclic systems that present in antibiotics such as penicillins and cephalosporins. Some substituted pyrazolines and their derivatives are used as antitumor, antibacterial, antifungal, antiviral, antiparasitic, anti-tubercular and insecticidal agents, some of these compounds have also anti-inflammatory, anti-diabetic, and anesthetic properties. Prompted by the above multi dimension observations, a research project was undertaken to synthesize a series of organophosphorous heterocycles baring azetadine-2-one, pyrazolone and Mannich base moieties in the same carbon skeleton structure.

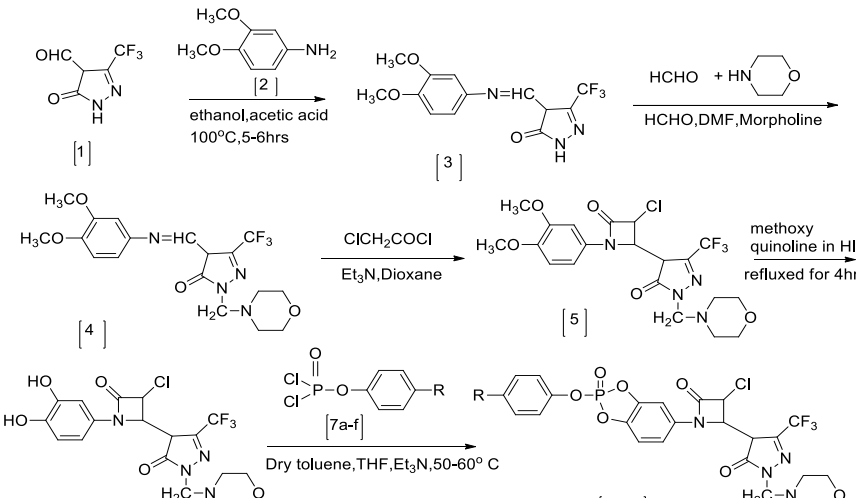


**Figure 1:**Scheme.1.4-(3-chloro-1-(2-(4-substitutedphenoxy)-2-oxidobenzo[d][1,3,2]dioxaphosphole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-ones(8a-f).

## 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 point was determined in open capillary tubes on Mel-Tempapparatus and is 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 13C-NMR spectra were recorded on a Varian XL-300 spectrometer operating at 400MHzfor 1H -NMR and 75 MHz for 13C-NMR. 31P-NMR spectra were recorded on a Varian XL-spectrometer

operating at161.89MHz. The compounds were dissolved in DMSO-d6 and Chemical shifts were referenced to TMS (1H and13C-NMR) and 85% H3PO4 (31P-NMR). Mass spectral data was recorded on FAB-MS instrument at 70ev with direct inlet system. Elemental analysis were recorded on a Carlo Erba 1108 elemental Analyser, Central Drug Research Institute, Lucknow, India [5].

### Preparation of Intermediates

**4-substituted phenyl phosphorodichloridates (7a-f):** Phosphorous oxychloride (15.3 gm,0.1 mole) in dry benzene (60ml) was taken into three-necked flasks (500 ml) equipped with a dropping funnel and reflux condenser fitted with a calcium chloride guard tube. The flask was heated and stirred by means of a hot plate-cum-magnetic stirrer. To this, dry triethylamine (10.1 gm, 0.1 mole) and dry benzene (50 ml) was added slowly and the reaction mixture was stirred for 30mits. To this mixture, freshly distilled phenol (9.4g ms, 0.1 mole) in dry benzene (60 ml) was added dropwise through the dropping funnel. The addition took about thirty minutes and the whole reaction mixture was refluxed with vigorous stirring for 10 hrs. The reaction mixture was cooled and solid triethylamine-hydrochloride was filtered off. The solvent from the filtrate was removed under reduced pressure in a rota evaporator. The dark brown liquid remained, was subjected to fractional distillation and the major product distilling at 118-124˚/11 mm was collected as a colorless glassy viscous liquid(8.3 gms,40%).

The other substituted phenylphosphorodichlorates (7a-f) were prepared by the same procedure by reacting equimolar quantities of phosphorous oxychloride and respectively substituted phenols in dry benzene in the presence of triethylamine [6].

## RESULTS AND DISCUSSIONS

### Synthesis of schiff's base 4-(((3,4–dimethoxyphenyl)imino)methyl)-3-(trifluoromethyl)-1H-pyrazol-5-(4H)-one (3)

A mixture of 5-oxo-3-(trifluoromethyl)-4,5-dihydro-1H-pyrazole-4-carbaldehyde(1,3.6 gms, 0.02 mol),anhydrous K2C03,3-4-dimethoxyaniline and DMF(50 ml) was stirred at room temperature for 8 hrs. The reaction mixture was diluted with ice-cold water. The separated solid was identified as 4-(((3,4-dimethoxyphenyl)imino)methyl)-3-(trifluoromethyl)-1-1H-pyrazol-5-(4H)-one.The light yellow precipitate was filtered off under vacuum and recrystallized from dimethylformamide, with a melting point of 146-148˚c, with a yield of (3,4.2 gms, 0.133 mol,75%).

### Synthesis of Mannich base 4-(((3,4–dimethoxyphenyl)imino)methyl)-1-(morpholino methyl)-3-(tri fluoromethyl)-1H-pyrazol-5(4H)-one(4)

Equimolar quantity of formaldehyde(1.2 gms.0.04 mol) and morpholine (3.5 gms, 0.04 mol) and 4-(((4-(((3,4-dimethoxyphenyl)imino)methyl)-3-(trifluoromethyl)-1-1H-pyrazol-5-(4H)-one(3, 6.3 gms,0.02 mol) were dissolved in absolute alcohol, to this three drops of acetic acid is added then heated on a steam bath for 5-6 hrs at 100˚c. After standing for 24hrs at room temperature, the product was dried and recrystallized from warm absolute alcohol. The separated solid was identified as 4-(((3,4-dimethoxyphenyl)imino)methyl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5-(4H)-one(4,5.8 gms,0.014 mol). The melting point of (4) was found to be 126-128˚c, with a yield of 70% [7].

### Synthesis of 4-(3-chloro-1-(3,4-dimethoxyphenyl)-4-oxoazetidin-2-yl)-1-(morpholino methyl)-3-(trifluoromethyl)-1H-pyrazol-5-(4H)-one(5)

Monochloroacetylchloride (1.7 gms, 0.015 mol) was added drop wise to the compound 4-(((3,4-dimethoxyphenyl)imino)methyl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5-(4H)-one(4, 4.2gms,0.01mol) and trimethylamine (0.02 mol) in dioxane (25 ml) at room temperature. The mixture was stirred for 8hrs and left at room temperature for 3days. Pour the contents on crushed ice to afford 4-(3-chloro-1-(3,4-dimethoxyphenyl)-4-oxoazetidin-2-yl)-1-(morpho linomethyl)-3-(trifluoromethyl)-1H-pyrazol-5-(4H)-one(5). The product thus formed was filtered and washed with sodium bicarbonate solution. The dried product was recrystallized with absolute alcohol. The separated solid was identified as 4-(3-chloro-1-(3,4-dimethoxyphenyl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5-(4H)-one(5,3.4 gms,0.0075 mol). The melting point was found to be 168-170˚c, with a yield of 75%.

**Synthesis of 4-(3-chloro-1-(3,4–dihydroxylphenyl)-4-oxaazetidin-2-yl)-1-(morpholino methyl)-3-(tri fluoromethyl)-1H-pyrazol-5(4H)-one**

A solution of hydroiodic acid (5%) was refluxed in glass joined apparatus for 3-4 hrs. The phenolic base 4-(3-chloro-1-(3,4–dihydroxyphenyl)-4-oxaazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoro methyl)-1H-pyrazol-5(4H)-one was liberated from the viscous hydroiodic acid by adding approximately the calculated amount of sodium carbonate solution, after neutralization, the reaction mixture was distilled under reduced pressure to afford crystallised product 4-(3-chloro-1-(3,4–dihydroxyphenyl)-4-oxaazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5 (4H)-one(6,3.0 gms,0.007 mol). The melting point of was found to be 184-186˚c, with a yield of 70%.

Synthesis [8] of 4-(3-chloro-1-(2-(4-substituted phenoxy)-2-oxido-benzo[d][1,3,2]dioxophos phole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoro methyl)-1H-pyrazol-5(4H)-one(8a-f).

A solution of phenylphosphorodichloridate(7a)(0.42 gms,0.002 mol) in 25 ml of dry toluene was added dropwise over a period of 20 mints to a stirred solution 4-(3-chloro-1-(3,4–dihydroxyphenyl)-4-oxaazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one(6,0.85 gms, 0.002 mol) and triethylamine (0.404gms, 0.004mol) in 30ml of dry toluene and 10ml of tetrahydro furan at 5˚c,after completion of the addition, the temperature of the reaction mixture was slowly raised to room temperature and stirred for 2 hrs. The reaction mixture was later heated to 50˚-60˚c and maintained for 4 hrs with stirring. The completion of the reaction was monitored by TLC analysis using n-hexane + ethyl acetate (7:3) as an elutent. Triethylamine hydrochloric acid was filtered from mixture and solvent was removed under reduced pressure. The residue was washed with water and then recrystallized from aqueous 2-propanol to get pure compound of 4-(3-chloro-1-(2-oxido-2-phenoxybenzene[d][1,3,2]dioxaphosphole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholino methyl)-3-(tri fluoromethyl)-1H-pyrazol-5(4H)-one (8a,0.63 gms, 0.001 mol), yield-52%, m.p 132-134˚c.

The similar procedure was adopted to synthesis (8a-f) from the reaction between (6) with p-tolylphosphorodichloridate(7b)/4-methoxyphenylphosphorodichloridate(7c)/4-chlorophenylphos phorodichloridate(7d)/4-bromophenylphosphorodichloridate(7e)/4-trifluorophenylphosphorodi chloridate(7f).

The structures of these newly synthesized compounds (8a-f) were characterized their elemental analysis and spectral data (IR, 1HNMR, 13C NMR, p31 NMR and Mass).

Physical, analytical and spectral data for the compounds:

**4-(((3,4–dimethoxyphenyl)imino)methyl)-3-(trifluoromethyl)-1H-pyrazol-5-(4H)-one**

IR (KBr)(ῡmax,$cm^{-1}$): 3225 $cm^{-1}$ (-N-H str. of pyrazoline-5-one),3040cm-1(Ar-H str.),1657$cm^{-1}$ (>C=O str. of pyrazoline-5-one),1620 $cm^{-1}$ (exocyclic azomethine >C=N-H str),1500,1430,1375 $cm^{-1}$ (str characteristic bands of pyrazoline-5-one ring),1340 $cm^{-1}$(C-F str band of CF3) and 1240 $cm^{-1}$(Ar-O-CH3 str). 1H NMR (400MHz, DMSO-d6):δ

2.20(d,1H,J=7.50,-CH of pyrazoline-5-one ring), 2.7(s,1H,-NH-of pyrazoline-5-one ring), 3.40 (s, 6H,two –OCH3 groups),6.9-7.2(m,3H of aromatic ring) and 8.4(d, 1H, J=7.50, >CH=N- exocyclic). Anal.Calcd.For C13H12N303F3 C 49.52%, H 3.80%, N, 13.33%. Found: C 48.8%, H 3.73%, N 12.95%.

4-(((3,4–dimethoxyphenyl)imino)methyl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one(4):

IR (KBr)(ūmax, $cm^{-1}$): 3040 $cm^{-1}$(Ar-H str.),2960,2870 $cm^{-1}$(str. of –CH2 group), 2500,1500,1000, 500 $cm^{-1}$ (characteristic absorption of morpholine ring),1657 $cm^{-1}$ (>C=O str. of pyrazoline-5-one), 1620cm-1(exocyclic azomethine >C=N-H str) 1500, 1430,1375$cm^{-1}$ (str. characteristic bands of pyrazoline-5-one ring),1340$cm^{-1}$ (C-F str. band of CF3),1240$cm^{-1}$ (Ar-O-CH3 str).1H NMR spectra (400MHz, DMSO-d6):δ2.20(d,1H,J=7.50,-CH of pyrazoline-5-one ring),2.45(t,4H,CH2 adj. N of morpholine), 3.65 (t,4H, CH2 adj.O- of morpholine), 3.40(s,6H,two –OCH3 groups),4.05(s,2H,N-CH2-N)6.9-7.2(m,3H of aromatic ring) and 8.4(d,1H,J=7.50,>CH=N- exocyclic). Anal.Calcd.For C18H21N4O4F3 C 52.17%, H 5.07%, N 13.52%. Found: C 51.5%, H 4.95%, N 13.09%.

**4-(3-chloro-1-(3,4-dimethoxyphenyl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoro methyl)-1H-pyrazol-5-(4H)-one**

IR (KBr)(ūmax, $cm^{-1}$)3040 $cm^{-1}$(Ar-H str.),2960,2870 $cm^{-1}$(str. of –CH2 group),2500,1500,1000, 500 $cm^{-1}$ (characteristic absorption of morpholine ring) ,1697 $cm^{-1}$, 1330 $cm^{-1}$, 651 $cm^{-1}$ (>C=O,C-N, C-Cl characteristic frequencies of 4-oxoazetidin ring),1657 $cm^{-1}$ (>C=O str. of pyrazoline-5-one), 1500,1430, 1375 $cm^{-1}$(str. of characteristic of pyrazoline-5-one ring),1340 $cm^{-1}$(C-F str. band of CF3),1240 $cm^{-1}$(Ar-O-CH3 str).1H NMR spectra (400 MHz, DMSO-d6 ) δ2.20(d,1H,J=7.50,-CH of pyrazoline-5-one ring),2.45(t,4H,N-Adj–CH2of morpholine),3.65 (t,4H, O-Adj –CH2 of morpholine), 3.40(s,6H,two –OCH3 groups),3.85(d,1H,-CH of azetidinone), 4.05(s,2H,N-CH2-N), 5.05(d,1H,-CH-Cl of azetidinone ring),6.9-7.2(m,3H of aromatic ring).Anal.Calcd.For C20H21N4O5F3 C 52.74%, H 4.83%, N 12.30%. Found: C 51.45%, H 4.68%, N 11.89%.

**4-(3-chloro-1-(3,4–dihydroxylphenyl)-4-oxaazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoro methyl)-1H-pyrazol-5(4H)-one**

IR (KBr)(ūmax, $cm^{-1}$) 3350 $cm^{-1}$(str. of intramolecular –OH band) 3040cm-1(Ar-H str.),2960, 2870 $cm^{-1}$(str. of –CH2 group),2500,1500,1000,500 $cm^{-1}$ (characteristic absorption of morpholine ring),1697 $cm^{-1}$, 1330 $cm^{-1}$,651cm $^{-1}$(>C=O,C-N,C-Cl characteristic frequencies of 4-oxoazetidin ring),1657 $cm^{-1}$ (>C=O str. of pyrazoline-5-one), 1500,1430, 1375 $cm^{-1}$(str. of characteristic of pyrazoline-5-one ring),1340 $cm^{-1}$(C-F str. band of CF3).1H NMR spectra (400 MHz, DMSO-d6 ) δ 2.20(d,1H,J=7.50,-CH of pyrazoline-5-one ring), 2.45(t,4H,N-Adj–CH2 of morpholine),3.65 (t, 4H,O-Adj –CH2 of morpholine), 3.85(d,1H,-CH of azetidinone one ring),4.05(s,2H,N-CH2-N),4.6 (s,2H,two phenolic –OH groups), 5.05(d,1H,-CH-Cl of azetidine one ring),6.9-7.2 (m,3H of aromatic ring). Anal.Calcd.For C18H18N4O5F3 C 50.5%, H 4.2%, N 13.1%. Found: C 49.4%, H 4.09%, N 12.76%.

**4-(3-chloro-1-(2-oxido-2-phenoxybenzene[d][1,3,2]dioxaphosphole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one(8a)**

IR (KBr)(ūmax, $cm^{-1}$)3040cm-1(Ar-H str.),2960,2870 $cm^{-1}$ (str.of–CH2 group),2500, 1500,1000, 500 (characteristic absorption of morpholine ring), 1697 $cm^{-1}$,1330 $cm^{-1}$,651 $cm^{-1}$(>C=O ,C-N,C-Cl characteristic frequencies of 4-oxoazetidin ring),1657 $cm^{-1}$(>C=O str. of pyrazoline-5-one), 1500,1430,1375( str. of characteristic Pyrazoline-5-one),1340 $cm^{-1}$(C-F str. band of CF3)1255 $cm^{-1}$ (P=O str. vibrations),954 $cm^{-1}$ (P-O str. vibration of P-O-C aromatic ring),1196 $cm^{-1}$ (Caromatic-O str. vibration of Caromatic-O-P group). 1H NMR spectra (400MHz, DMSO-d6):δ2.20(d,1H,J=7.50Hz,-CH of pyrazoline-5-one),2.45(t,4H,N-Adj,-CH2 of morpholine),3.65

(t,4H,O-Adj,-CH2 of morpholine), 3.85(d,1H,-CH azetidinone ring), 4.05(s,2H,N-CH2-N),5.05(d,1H,CH-Cl of azetidinone ring) [9,10].

### 4-(3-chloro-1-(2-oxido-2-(p-tolyloxy)benzo[d][1,3,2]dioxaphosphole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(tri fluoro methyl)-1H-pyrazol-5(4H)-one(8b)

IR (KBr)(ūmax, $cm^{-1}$) 3040cm-1(Ar-H str.),2960,2870 $cm^{-1}$ (str.of–CH2 group),2500,1500,1000,500 (characteristic absorption of morpholine ring), 1697 $cm^{-1}$,1330 $cm^{-1}$,651 $cm^{-1}$(>C=O ,C-N,C-Cl characteristic frequencies of 4-oxoazetidin ring),1657 $cm^{-1}$(>C=O str. of pyrazoline-5-one),1500, 1430,1375( str. of characteristic Pyrazoline-5-one),1340 $cm^{-1}$(C-F str. band of CF3)1239 $cm^{-1}$(P=O str. vibrations),960 $cm^{-1}$ (P-O str. vibration of P-O-C aromatic ring),1192 $cm^{-1}$ (Caromatic-O str. vibration of Caromatic-O-P group). 1H NMR spectra (400MHz, DMSO-d6): δ 2.20(d,1H,J=7.50Hz,-CH of pyrazoline-5-one),2.45(t,4H,N-Adj,-CH2 of morpholine),3.65(t,4H,O-Adj,-CH2 of morpholine),3.85(unequal quartet, 1H,-CH of azetidinone ring),4.05(s,2H,N-CH2-N),5.05 (d, 1H, J=8.5Hz, CH-Cl of azetidinone ring), 6.9-7.10(m,7H, C6H4 and C6H3 rings),2.34(s, 3H,-CH3). 13C NMR (75 MHz, DMSO-d6):δ 155.6,23.0,175.9,56.6,56.8,161.9,135.7,107.7,145.4,140.8,117.5, 115.6,150.2,120.3,130.1,121.3,130.1,120.3,70.3,53.2,66.4,66.4,53.2,125.8,21.3 corresponding to C1,C2,C3,C4,C5, C6,C7,C8, C9,C10,C11, C12, C13, C14,C15, C16,C17,C18,C19,C20,C21, C22,C23,C24, C25. 31P –NMR (161.89 MHz, DMSO-d6): δ -8.48 ppm. Anal. Calcd. For C25H23N4O7PClF3 C 48.7%, H 3.73%, N 9.09%. Found: C 48.1%, H 3.63%, N 8.89%.

### 4-(3-chloro-1-(2-(4-methoxyphenoxy)-2-oxidobenzo[d][1,3,2]dioxaphosphol-5-yl)-4-oxo azetidin-2-yl)-1-(morpholinomethyl)-3-(trifloromethyl)-1H-pyrazol-5-(4H)-one(8c)

IR (KBr)(ūmax, $cm^{-1}$) 3040 $cm^{-1}$(Ar-H str.),2960,2870 $cm^{-1}$ (str.of–CH2 group),2500, 1500,1000,500 (characteristic absorption of morpholine ring), 1697cm-1,1330 $cm^{-1}$,651 $cm^{-1}$(>C=O ,C-N,C-Cl characteristic frequencies of 4-oxoazetidin ring),1657 $cm^{-1}$(>C=O str. of pyrazoline-5-one),1500, 1430,1375( str. of characteristic Pyrazoline-5-one),1340 $cm^{-1}$(C-F str. band of CF3)1245 $cm^{-1}$(P=O str. vibrations),944 $cm^{-1}$ (P-O str. vibration of P-O-C aromatic ring),1184 $cm^{-1}$ (Caromatic-O str. vibration of Caromatic-O-P group). 1H NMR spectra (400MHz, DMSO-d6): δ 2.20(d,1H,J=7.50Hz,-CH of pyrazoline-5-one),2.45(t,4H,N-Adj,-CH2 of morpholine),3.65(t,4H,O-Adj,-CH2 of morpholine),3.85(unequal quartet, 1H,-CH of azetidinone ring),4.05(s,2H,N-CH2-N),5.05(d,1H, J=8.5Hz, CH-Cl of azetidinone ring), 6.8-7.0(m,7H,C6H4 and C6H3 rings),3.4(s, 3H,-OCH3). 13C NMR (75 MHz, DMSO-d6):δ 155.6,23.0,175.9,56.6,56.8,161.9,135.7,107.7,145.4,140.8,117.5, 115.6,142.5,116.9,115.7, 153.2,115.7,116.9,70.3,53.2,66.4,66.4,53.2,125.8,55.8 corresponding to C1,C2,C3,C4,C5,C6,C7,C8,C9,C10,C11,C12,C13,C14,C15,C16,C17,C18,C19,C20,C21,C22,C23,C24,C25.31PNMR (161.89 MHz, DMSO-d6): δ -8.92ppm. Anal.Calcd.For C25H23N4O8PClF3 C 47.50%, H 3.64%, N 8.86%. Found: C 46.9%, H 3.56%, N 8.63%.

### 4-(3-chloro-1-(2-(4-chlorophenoxy)-2-oxidobenzo[d][1,3,2]dioxaphosphol-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(tri fluoromethyl)-1H-pyrazol-5(4H)-one(8d)

IR (KBr)(ūmax, $cm^{-1}$) 3040 $cm^{-1}$(Ar-H str.),2960,2870 $cm^{-1}$ (str.of–CH2 group),2500, 1500,1000,500 (characteristic absorption of morpholine ring), 1697 $cm^{-1}$,1330 $cm^{-1}$,651 $cm^{-1}$(>C=O ,C-N,C-Cl characteristic frequencies of 4-oxoazetidin ring),1657 $cm^{-1}$(>C=O str. of pyrazoline-5-one),1500, 1430,1375( str. of characteristic Pyrazoline-5-one),1340 $cm^{-1}$(C-F str. band of CF3)1270 $cm^{-1}$(P=O str. vibrations),969 $cm^{-1}$ (P-O str. vibration of P-O-C aromatic ring),1210 $cm^{-1}$ (Caromatic-O str. vibration of Caromatic-O-P group). 1H NMR spectra (400 MHz, DMSO-d6): δ 2.20(d,1H,J=7.50Hz,-CH of pyrazoline-5-one),2.45(t,4H,N-Adj,-CH2 of morpholine),3.65(t,4H,O-Adj,-CH2 of

morpholine),3.85(unequal quartet, 1H,-CH of azetidinone ring), 4.05(s,2H,N-CH2-N),5.05(d,1H, J=8.5Hz, CH-Cl of azetidinone ring), 7.20-7.40(m,7H,C6H4 and C6H3 rings). 13C NMR (75 MHz, DMSO-d6):δ155.6,23.0,175.9,56.6,56.8,161.9,135.7,107.7,145.4,140.8,117.5,115.6,142.5,116.9, 115.7,153.2,115.7,116.9,70.3,53.2,66.4,66.4,53.2,125.8 corresponding to C1,C2,C3,C4,C5, C6,C7,C8, C9,C10,C11,C12,C13,C14,C15,C16,C17,C18,C19,C20,C21,C22,C23,C24. 31P–NMR (161.89MHz, DMSO-d6): δ -7.75ppm. Anal.Calcd. For C24H21N4O7PCl2F3 C 45.2%, H 3.29%, N 8.79%. Found: C 44.6%, H 3.19%, N 8.56%.

**4-(1-(2-(4-bromophenoxy)2-oxidobenzo[d][1,3,2]dioxaphosphol-5-yl)-3-chloro-4-oxoazetidin-2-yl)-1-(morpho linomethyl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one(8e)**

IR (KBr)(ῡmax, $cm^{-1}$) 3040 $cm^{-1}$(Ar-H str.),2960,2870 $cm^{-1}$ (str.of–CH2 group),2500, 1500,1000,500 (characteristic absorption of morpholine ring), 1697 $cm^{-1}$,1330 $cm^{-1}$,651 $cm^{-1}$(>C=O ,C-N,C-Cl characteristic frequencies of 4-oxoazetidin ring),1657 $cm^{-1}$(>C=O str. of pyrazoline-5-one), 1500,1430,1375( str. of characteristic Pyrazoline-5-one),1340 $cm^{-1}$(C-F str. band of CF3)1265 $cm^{-1}$ (P=O str. vibrations),963 $cm^{-1}$ (P-O str. vibration of P-O-C aromatic ring),1203 $cm^{-1}$ (Caromatic-O str. vibration of Caromatic-O-P group). 1H NMR spectra (400MHz, DMSO-d6): δ 2.20 (d,1H,J=7.50Hz,-CH of pyrazoline-5-one),2.45(t,4H,N-Adj,-CH2 of morpholine),3.65(t,4H,O-Adj,-CH2 of morpholine),3.85(unequal quartet, 1H,-CH of azetidinone ring),4.05(s,2H,N-CH2-N),5.05(d,1H, J=8.5Hz, CH-Cl of azetidinone ring), 7.10-7.30(m,7H, C6H4 and C6H3 rings). 13C NMR (75 MHz, DMSO-d6):δ155.6,23.0,175.9,56.6,56.8,161.9,135.7,107.7,145.4,140.8,117.5,115.6,142.5,116.9, 115.7,153.2,115.7,116.9, 70.3,53.2,66.4,66.4,53.2,125.8 corresponding to C1, C2, C3, C4,C5, C6,C7, C8,C9,C10,C11,C12,C13,C14,C15,C16,C17,C18,C19,C20,C21,C22,C23,C24. 31P–NMR (161.89 MHz, DMSO-d6): δ -8.10 ppm. Anal.Calcd.For C24H21N4O7PClF3Br C 42.32%, H 3.08%, N 8.22%. Found: C 41.72%, H 2.99%, N 7.99%.

**4-(3-chloro-1-(2-oxido-2-(4-(trifluoromethyl) phenoxy)benzo[d][1,3,2]dioxaphosphol-5-yl)-4-oxoazetidin-2-yl)-1-(morpho linomethyl)-3-(trifluoro methyl)-1H-pyrazol-5-(4H)-one(8f)**

IR (KBr)(ῡmax, $cm^{-1}$) 3040 $cm^{-1}$(Ar-H str.),2960,2870 $cm^{-1}$ (str.of–CH2 group),2500, 1500,1000,500 (characteristic absorption of morpholine ring), 1697 $cm^{-1}$,1330 $cm^{-1}$,651 $cm^{-1}$(>C=O ,C-N,C-Cl characteristic frequencies of 4-oxoazetidin ring),1657 $cm^{-1}$(>C=O str. of pyrazoline-5-one), 1500, 1430,1375( str. of characteristic Pyrazoline-5-one),1340 $cm^{-1}$(C-F str. band of CF3)1276 $cm^{-1}$ (P=O str. vibrations),975 $cm^{-1}$ (P-O str. vibration of P-O-C aromatic ring),1213 $cm^{-1}$ (Caromatic-O str. vibration of Caromatic-O-P group). 1H NMR spectra (400 MHz, DMSO-d6): δ 2.20(d,1H,J=7.50Hz,-CH of pyrazoline-5-one),2.45(t,4H,N-Adj,-CH2 of morpholine),3.65(t,4H,O-Adj,-CH2 of morpholine),3.85(unequal quartet, 1H,-CH of azetidinone ring),4.05(s,2H,N-CH2-N),5.05(d,1H, J=8.5Hz, CH-Cl of azetidinone ring),6.9-7.2(M,3H,aromatic ring),7.30-7.50(M,7H of C6H3 and C6H4 rings). 13C NMR (75 MHz, DMSO-d6):δ 155.6,23.0,175.9,56.6,56.8,161.9,135.7,107.7, 145.4,140.8,117.5,115.6,142.5,116.9,115.7,153.2,115.7,116.9,70.3,53.2,66.4,66.4,53.2,155.8, 124.1 corresponding to C1,C2,C3,C4,C5,C6,C7,C8,C9,C10,C11,C12,C13,C14,C15,C16,C17,C18,C19,C20,C21,C22, C23,C24,C25. 31P–NMR (161.89MHz, DMSO-d6):δ -7.56ppm. Anal.Calcd.For C25H20N4O7PClF6 C 44.80%, H 2.98%, N 8.36%. Found: C 44.2%, H 2.88%, N 8.15%.

**Biological activity:** The antimicrobial activity of these newly synthesized compounds was performed according to disc diffusion method, as recommended by the National Committee for Clinical Laboratory [11-15]. The synthesized compounds were used at the concentration of 250 µg/ml DMF as a solvent [16].

**Antibacterial activity:** The antibacterial activity of 4-(3-chloro-1-(2-oxido-2-(4-substituted phenoxy)[d][1,3,2]di oxaphosphole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5 (4H)-one(8a-f) were screened against the staphylococcus aureus NCCS 2079( SA), Bacillus cereus NCCS 2106(BC)(gram positive) and Escherichia coli NCCS 2065(EC), and Pseudomonas aeruginosa NCCS 2200(PA)(gram negative) organisms. Most of the compounds exhibit moderate antibacterial activity against both bacteria. The presence of –CF3 (8f), chloro (-Cl, 8d) and bromo (-Br, 8e) showed more activity than other substituted compounds. The order of antibacterial activity is 8f>8d>8e>8c>8b>8a.

**Table 1:** Antibacterial activity of 4-(3-chloro-1-(2-oxido-2-(4-substituted phenoxy)[d][1,3,2]dioxaphos phole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one(8a-f).

| | | | Zone of inhibition(mm) | | | |
|---|---|---|---|---|---|---|
| S.NO | COMPOUND | R | *staphylococcus aureus NCCS 2079* | *Bacillus cereus NCCS 2106* | *Escherichia coli NCCS 2065* | *Pseudomonas aeruginosa NCCS 2200* |
| | | | *250 µg/ disc* | *250 µg/ disc* | *250 µg/ disc* | *250 µg/ disc* |
| | | | | | | |
| 1 | 8a | H | 11 | 12 | 10 | 11 |
| 2 | 8b | $CH_3$ | 9 | 11 | 7 | 9 |
| 3 | 8c | $OCH_3$ | 17 | 18 | 16 | 17 |
| 4 | 8d | Cl | 15 | 16 | 13 | 14 |
| 5 | 8e | Br | 16 | 17 | 14 | 15 |
| 6 | 8f | $CF_3$ | 14 | 13 | 12 | 12 |
| Amoxicillin | | | 22 | 25 | 21 | 23 |

**Antifungal activity:** The antifungal activity of 4-(3-chloro-1-(2-oxido-2(4-substituted phenoxy)[d][1,3,2]dioxaphos phole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one (8a-f) were screened against the Aspergillus Niger NCCS 1196 (AN) and candied albicans NCCS 3471(CA) organisms. Most of the compounds exhibit moderate antifungal activity against both fungi. The presence of -CF3(8f), chloro(8d) and bromo(8e) showed more activity than other substituted compounds. The order of antifungal activity is 8f>8d>8e>8c>8b>8a [17].

**Table 2:** Antifungal activity of 4-(3-chloro-1-(2-oxido-2-(4-substituted phenoxy)[d][1,3,2]dioxaphosphole-5-yl)-4-oxoazetidin-2-yl)-1-(morpholinomethyl)-3-(trifluoromethyl)-1H-pyrazol-5(4H)-one(8a-f).

| | | | Zone of inhibition(mm) | |
|---|---|---|---|---|
| S.NO | COMPOUND | R | *Aspergillus niger NCCS 1196* | *candied albicans NCCS 3471* |
| | | | *250 µg/ disc* | *250 µg/ disc* |
| | | | | |
| 1 | 8a | H | 12 | 11 |
| 2 | 8b | $CH_3$ | 10 | 9 |
| 3 | 8c | $OCH_3$ | 20 | 18 |
| 4 | 8d | Cl | 15 | 15 |
| 5 | 8e | Br | 17 | 17 |
| 6 | 8f | $CF_3$ | 13 | 11 |
| Ketaconazole | | | 22 | 25 |

The docking studies of 8a, 8b, 8c, 8d, 8e, 8f were carried out as model compounds on sortase-A enzyme. The docking ligands were found to have some interactions between an oxygen atom of the ligands and sortase-A enzyme. The results pertaining to antimicrobial docking studies were shown in the Table 1-2 and fig 1. Moreover, these docked conformations form hydrogen bond interactions with the active site of the enzyme. The common hydrogen bonding interactions were formed between all the docked ligands and ILE57PDB:1H, TYR54H, THR77H. Except 8e, the remaining Mannich bases show two hydrogen bonds with sortase-A enzyme. The order of enzyme-ligand hydrogen bond energy (S(Hb_ext)) is 8b>8f>8e>8d>8a=8c. The vanderwaals interactions between ligand-enzyme were also noticed. The order of enzyme-ligand vanderwaals score of interaction was found to be 8d>8f>8e>8c>8a>8b. However the ligands fail to exhibit intramolecular hydrogen bonding with the enzyme. The ligands exhibit minimum intramolecular strain. Finally, all the ligands exhibit moderate to good antibacterial activity with Sortase-A enzyme. The order of gold score fitness value of the ligands is 8e>8c>8f>8d>8a>8b. According to gold score fitness value ligand 8e exhibits high binding activity with the enzyme and ligand 8b showed leads binding activity with the enzyme [18].

## CONCLUSION

In the present investigations, we report herein synthesis of novel benzodioxaphosphole-2-oxide derivatives containing structurally varied heterocycles. The salient observations noticed in the experimental results and structural elucidations were briefly described in this research paper. This research article gives information about abstract of work done besides highlights in the results and discussion. The spectral data (IR, 1H NMR, 13C NMR, P31-NMR and Mass), antimicrobial profile and docking studies were presented in this research paper. The piece of novel work on “Synthesis, Characterization, Biological Evaluation and Docking studies of (8a-f) add new dimensions to the Organophosphorous Chemistry.

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## REFERENCES

1. John-Hopkins, et al. Pancreas: Anatomy and Functions. 2020.
2. Nandy D, et al. Growth factor mediated signaling in pancreatic Pathogenesis. Cancer. 2011;3:841-871.
3. Dennis LK, et al. Harrison's Principles of Internal Medicine, Nineteenth ed. McGraw Hill Education Medical. New York. 2015.
4. Le Bras S, et al, Fibroblast growth factor 2 promotes pancreatic epithelial cell proliferation via functional fibroblast growth factor receptors during embryonic life. Diabete. 1998;1236–1242.
5. Edlund E, Pancreatic organogenesis—Developmental mechanisms and implications for therapy. Nat Rev Genet. 2002;3:524-532.
6. Yamanaka Y, et al. Overexpression of acidic and basic fibroblast growth factors in human pancreatic cancer correlates with advanced tumor stage. Cancer Res. 1993;53:5289-5296..
7. Johnson DE. Diverse forms of a receptor for acidic and basic fibroblast growth factors. Mol Cell Biol. 1990;10:4728-4736.
8. Jaye M, et al. Fibroblast growth factor receptor tyrosine kinases: Molecular analysis and signal transduction. Biochim Biophys Acta. 1992;1135:185-199.
9. Mignatti P, et al. *In vitro* angiogenesis on the human amniotic membrane: Requirement for basic fibroblast growth factor-induced proteinase. J Cell Biol. 1989;108:671-682.
10. Moscatelli D, et al. Purification of a factor from human placenta that stimulates capillary endothelial cell protease production, DNA synthesis, and migration.ProcNatlAcad Sci. 1986;83:2091-2095.
11. Montesano R, et al. Basic fibroblast growth factor induces angiogenesis *in vitro*. ProcNatlAcad Sci. 1986;83:7297-7301.
12. Abadpour S, et al. Glial cell-line derived neurotrophic factor protects human islets from nutrient deprivation and endoplasmic reticulum stress induced apoptosis. Sci Rep. 2017;7:1575.
13. Mwangi S, et al. Glial cell line-derived neurotrophic factor increases beta-cell mass and improves glucose tolerance. Gastroenterol.2008;134:727-737.
14. Heuckeroth RO, et al. Neurturin and GDNF promote proliferation and survival of enteric neuron and glial progenitors *in vitro.* Dev Biol. 1998;200:116-129.
15. BalohRHet al. The GDNF family ligands and receptors -implications for neural development. CurrOpinNeurobiol. 2000;10:103-110.
16. Seaberg RM, et al. Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nat Biotechnol. 2004;22:1115-1124.
17. Pierucci D, et al. NGF-withdrawal induces apoptosis in pancreatic beta cells *in vitro*, Diabetologia. 2001;44:1281-1295.
18. Teitelman G, et al. Islet injury induces neurotrophin expression in pancreatic cells and reactive gliosis of peri-islet Schwann cells. J Neurobiol. 1998;34:304-318.