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RESEARCH ARTICLE

ORGANOPHOSPHORUS COMPOUNDS CONTAINING 3-(SUBSTITUTED PHENYL)- 2-THIOXOIMIDAZOLIDIN-4-ONE AS POTENT ANTIMICROBIAL AGENTS

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Abstract

A series of biologically active organophosphorus compounds have been synthesized by the reactions of 4-Chlorophenyl dichlorophosphate with 3-(substitutedphenyl)-2-thioxoimidazolidin-4-one. The compounds have been characterized on the basis of elemental analyses and spectral (IR, ^{1}H NMR ^{31}P NMR) data. All the compounds were screened for their antimicrobial activity .They were found to possess significant anti-microbial activity.

Introduction:-

Thiohydantoins are sulfur analogs of hydantoins with one or both carbonyl groups replaced by thiocarbonyl groups, (Johnson et al., 1913). The thiohydantoins nucleus is a 5-membered ring system containing a reactive cyclic thiourea core. Among the known thiohydantoins, 2-thiohydantoins are most notably known due to their wide applications as hypolipidemic, (Tompkins, et al., 1986), anticarcinogenic (Al-Obaid et al.,1996), antimutagenic(Takahashi et al . 1998), antithyroidal (Marx, et al., 1970), antiviral (e.g., against herpes simplex virus, HSV) (El-Barbary, et al., 1994), human immunodeficiency virus (HIV)(Chérouvrier, et al 2004) and tuberculosis (Archer et al., 1956), antimicrobial (antifungal and antibacterial) (Lacroix et al.,2000), anti-ulcer, anti-inflammatory agents (Curran et al., 1976) antioxidant agents (Camargo, et al., 2021) as well as pesticides (Nagpal et al., 1984). Additionally, 2-thiohydantoins have been used as reference standards for the development of C-terminal protein sequencing (Mo et al.,1997), as reagents for the development of dyes (Nelson et al.,1997) and in textile printing, metal cation complexation and polymerization catalysis (Kandil et al.,2004) . Some thiohydantoins can be used as cardio protective agents for the prevention of atherosclerosis in man (Elokdah et al.,2004). Lambert et al. have proved the human CB_1 and CB_2 cannabinoid receptor affinity of the thiohydantoins (Muccioli 2005a,b.,2006).

On the other hand the chemistry of organophosphorus heterocyclic compounds has always attracted much attention because of their unique potential biological properties. A few recent studies (Sengupta et al.,1998a,b; 2000,2002,2003, Chandra et al.,2003, 2005, Pandey et al.,2012) have shown that on the basis of suitable logic organic molecules, incorporating phosphorus may be designed such that they may be less dangerous in use without losing their value as effective pesticides. The discovery of the mechanism of action (Wang, 1998) of organophosphorus compounds made it possible to develop the fundamental principles of the directed synthesis of new substances and to establish the cause of their selective action on an organism. Studies on organophosphorus derivatives could constitute a new and promising field of application in the national economy.

In this section reactions of 4-chlorophenyl dichlorophosphate with 3-(substituted phenyl)-2-thioxoimidazolidin-4-one are reported. The anti-microbial efficacy of these newly synthesized organophosphorus compounds against

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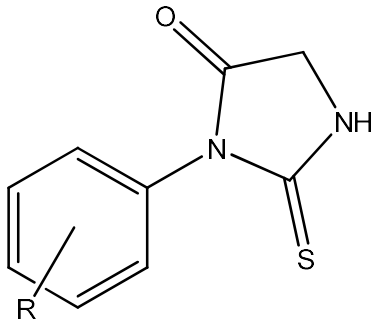
various important bacterial and fungal pathogens were also evaluated. The preliminary bioassay tests showed that the synthesized compounds exhibited a significant antimicrobial activity. The structures of various thiohydantoins used for the study are shown below**.**



Where,
R= 3-Cl (I), 4-Br (II), 4-F (III), 4-NO_2 (IV), 2,4-Cl_2 (V), 2,5-Cl_2 (VI), 2-OCH_3,4-NO_2 (VII), 4-OCH_3,2-NO_2(VIII), 3,4-$(CH_3)_2$(IX).

Results and Discussion:-

Reactions of 4-Chlorophenyldichlorophosphate with 3-(substituted phenyl)-2-thioxoimidazolidin-4-one ligands have been carried out in benzene in the presence of pyridine and a variety of organophosphorus derivatives have been isolated according to **scheme**. The methods used for the preparation and isolation of these compounds gave materials of good purity as supported by their analyses and TLC. The elemental analyses and physical properties of the organophosphorus compounds are given in **Table 1**. All compounds are quite stable in air. The organophosphorus derivatives are found to be soluble in dimethylformamide, tetrahydrofuran and dimethylsulfoxide. All of these compounds are cream to yellow in colour. The compounds melt in the temperature range of 105-168ºC.

Infrared spectra

A comparison of the characteristic infrared absorption bands of 3-(substituted phenyl)-2-thioxoimidazolidin-4-one with those of the corresponding organophosphorus derivatives reveals the following important features:

1. The infrared spectra of thiohydantoins show sharp bands at ca. 3250 cm^{-1} and 1250 cm^{-1}, which may be assigned (Nakamoto 1970) to ν(N-H) and δ(N-H) vibrations, respectively. However, in the spectra of organophosphorus derivatives these bands disappear, indicating the displacement of N-H hydrogen by phosphorus. The formation of phosphorus-nitrogen bond is also confirmed by the appearance of bands at ca.730-750 cm-1 assignable (Giri 1977) to ν(P-N).
2. The spectra of thiohydantoins show strong bands at ca. 1680 cm-1 and 1150 cm-1 assignable to (Sengupta et al.,1999) to ν(C=O) and ν(C=S) vibrations, respectively. These bands appear almost at the same position in the spectra of organophosphorus derivatives.
3. In addition the organophosphorus derivatives (I, II, III, IV, V, VI, VII, VIII, IX) show bands at ca.1290-1310 cm^{-1} assignable to ν(P=O) (Bradley 1969, Ewald 1969) vibrations. Same derivatives also show bands at ca. 960 cm^{-1} and 1240 cm^{-1} assignable to ν (P-O) and ν (O-C) aromatic respectively.

Nuclear Magnetic Resonance Spectra

The ^1H NMR spectra were recorded on a Bruker Avance III, 400MHz spectrometer operating at 400 MHz to ^1H and 161.9 MHz for ^{31}P NMR using DMSO-d_6 as solvent. In general, a slight shift to lower field in the position of the resonance signals of various protons in the organophosphorus derivatives was observed due to a change in the electronic- environment (de-shielding) around protons in the thiohydantoins. Of course, the protons of R groups in the thiohydantoins are affected very little due to the remote positions of these protons from the phosphorus atom. The signals due to aromatic ring protons appear in region ca. δ 6.58-8.65. The signals due to N–H protons appears at about ca. δ 4.8-4.9 in the spectra of all thiohydantoins ligands which disappears in their corresponding organophosphorus derivatives indicating the deprotonation of N–H proton and formation of bond between nitrogen and phosphorus.

^{31}P NMR chemical shifts of the compounds (I, II, III, IV, V, VI, VII, VIII, IX) appeared in the region--18.21ppm .

Antimicrobial Activity

Antimicrobial tests were performed on two bacteria (*Staphylococcus aureus and Escherichia coli*) and three fungus (*Aspergillus niger, Aspergillus ochraceus and Fusarium oxyporum)*. The media used were prepared by dissolving separately 2g of the nutrient broth powder and 38g of the Mueller Hinton agar powder in 250mL and 1L of deionized water, respectively. The two media were sterilized in an autoclave at 121ºC for 15 min and then stored overnight in a refrigerator after cooling. Cultures of the microorganisms were prepared in sterile nutrient broth and incubated for 24 h at 37ºC for the bacteria and 27ºC for the fungi. 0.1mL of each of the overnight cultures in sterile test tubes with caps were made upto 10 mL with 9.9mL of sterile deionized water. The technique used for the study was agar-well diffusion.

Solutions of concentrations 250, 500 and 1000 ppm were made in methanol. Methanol was also used as the negative control. The positive controls for bacteria and fungi were discs of commercial antibiotics Streptomycin and Griseofulvin respectively dissolved in methanol. The discs were carefully placed on the inoculated media with the aid of sterile forceps. The plates inoculated with bacteria were incubated at 37ºC for 24 h and those inoculated with fungi were incubated at 27ºC for 72 h. Afterwards, the zones of inhibition of microbial growth that appeared around the wells of the compounds were examined and the diameters measured and recorded in millimetres (mm). Antimicrobial activities of newly synthesized all organophosphorus compounds was evaluated in vitro against Gram positive bacteria- Staphylococcus aureus and Gram negative bacteria- Escherichia coli (**Table 2**). The majority of the compounds (I-IX) exhibited moderate to good against both the bacteria. The same compounds were screened for their antifungal activity (**Table 3**) against A. niger, A. ochraceus and F. oxyporum species. It is gratifying to observe that the majority of the compounds (I-IX) exhibited moderate to good antifungal activity when compared with the Griseofulvin in reference.

Materials and Methods:-

The reactions of 4-Chlorophenyldichlorophosphate with 3-(substituted phenyl)-2-thioxoimidazolidin-4-one ligands were carried out under inert atmosphere and anhydrous conditions. Special precautions were taken to exclude moisture from the apparatus and the starting materials (4-Chlorophenyldichlorophosphate) as reactions were susceptible to hydrolysis. Glass apparatus with interchangeable joints were used throughout the work. All the organic solvents used were of analytical reagent grade. The solvents were purified and dried using the method described in the literature (Chaturvedi 1995). 4-Chlorophenyldichlorophosphate was procured from Aldrich Chemical Company, Inc. USA and was used without further purification**.** The details of analysis and physical measurements were the same as reported earlier (Sengupta 1999).

Experimental

General procedure for the synthesis of 3-(substituted phenyl)-2-thioxoimidazolidin-4-one

Synthesis of 3-(substituted phenyl)-2-thioxoimidazolidin-4-one involves two steps:

Synthesis of 1-(substituted phenyl) thiourea

To a reaction mixture of benzoyl chloride (0.1 mol) and ammonium thiocynate (0.1 mol) in acetone (25 mL), appropriate amine in case of solid amine, acetone solution of amine) (0.1 mol) was added slowly. The resulting mixture was refluxed gently for 10-15 min. The mass was poured in to water. Precipitate thus, obtained, was filtered, dissolved in boiling alkali solution and filtered. To the filtrate, a little amount of water was added. Then it was acidified and was made slightly basic with ammonia. On cooling, the crystal of thiourea were precipitated out.

Synthesis of 3-(substituted phenyl)-2-thioxoimidazolidin-4-one

A mixture of appropriate thiourea (0.3 mol) and monochloroacetic acid (0.2 mol) in pyridine (30mL) was refluxed for 4 h. After cooling and pouring in to water, the required thiohydantoin precipitated out, which was filtered off and re-crystallised from methanol.Yield : 65-70%.

General procedure for the synthesis of organophosphorus compounds (I-IX):

The organphosphorus compounds were prepared by mixing 4-Chlorophenyl dichlorophosphate (1 mol) and the appropriate ligand 3-(substituted phenyl)-2-thioxoimidazolidin-4-one (2 mol) in benzene (30mL) in presence of pyridine (2 mol) with continuous stirring .Stirring was continued at room temperature over a period of 7-10 h under

anhydrous conditions. After completion of reaction, the reaction mixture was put into a beaker containing crushed ice. A solid was obtained. It was collected and re-crystallised from acetone .For the sake of brevity, the details of the individual reactions along the physical characterization are given in **Table 1**.

Chlorophenyl bis(3-(3-chloro phenyl)-4-oxo-2-thioxoimidazolidin-1-yl) phosphinate (I) : Yield: 64%, m.p.: 105-107°C, IR (KBr,cm^{-1}): 3069 (C–H $_{aro,str.}$),1672cm-1 (C=$O_{str.}$), 1481.38cm-1 (C=C $_{aro,str.}$), 1140 cm-1 (C=S $_{aro,str.}$), 1280 (P=O), 961 (P-O), 1240 (O-C $_{aro,str}$), 730 (P-N), 728 cm-1 (3-C-Cl).767 cm-1 (4-C-Cl). $^{1}H_{str.}$NMR (DMSO-d_6 ,δ) : 6.89-7.99 (m ,12H, Ar-H), 4.01 (s, 4H, CH_2). ^{31}P NMR (DMSO-d_6 ,δ): -18.63 . Anal. Found (Calcd)% for $C_{24}H_{16}O_4N_4S_2PCl_3$: C, 45.8(46.0); H, 2.3(2.5); N, 8.7(8.9); S, 10.0(10.2); Cl, 16.7(17.0) .

Chlorophenyl bis(3-(4-bromophenyl)-4-oxo-2-thioxoimidazolidin-1-yl)phosphinate (II): Yield: 60%, m.p.:132-134°C, IR (KBr,cm^{-1}): 3068(C–H $_{aro,str.}$),1686cm-1 (C=$O_{str.}$), 1490cm-1 (C=C $_{aro,str.}$), 1148 cm-1 (C=S $_{aro,str.}$), 1298 (P=O), 965 (P-O), 1248 (O-C $_{aro,str}$), 740 (P-N), 770 cm-1 (C-Cl), 779cm-1(C-Br $_{aro,str.}$). $^{1}H_{str.}$NMR (DMSO-d_6 ,δ) : 6.89-8.72 (m ,12H, Ar-H), 4.39 (s, 4H, CH_2). ^{31}P NMR (DMSO-d_6 ,δ): -18.63 . Anal. Found (Calcd)% for $C_{24}H_{16}O_4N_4S_2PClBr_2$: C, 40.1(40.3); H, 2.0(2.2); N, 7.5(7.8); S, 8.6(8.9) Cl, 4.7(4.9); Br, 22.0(22.3).

Chlorophenyl bis(3-(4-fluorophenyl)-4-oxo-2-thioxoimidazolidin-1-yl) phosphinate(III) : Yield: 602%, m.p.:134-136°C, IR (KBr,cm^{-1}): 3063(C–H $_{aro,str.}$),1744cm-1 (C=$O_{str.}$), 1489cm-1 (C=C $_{aro,str.}$), 1244 cm-1 (C=S $_{aro,str.}$), 1292 (P=O), 964 (P-O), 1243 (O-C $_{aro,str}$), 734 (P-N), 768 cm-1 (C-Cl), 779cm-1(C-F $_{aro,str.}$). $^{1}H_{str.}$NMR (DMSO-d_6 ,δ) : 6.89-8.63 (m ,12H, Ar-H), 4.39 (s, 4H, CH_2). ^{31}P NMR (DMSO-d_6 ,δ): -18.63 . Anal. Found (Calcd)% for $C_{24}H_{16}O_4N_4S_2PCl\,F_2$: C, 48.2(48.5); H, 2.3(2.6); N, 9.2(9.4); S, 10.6(10.8) Cl, 5.7(5.9); F, 6.2(6.4).

Chlorophenyl bis(3-(4-nitrophenyl)-4-oxo-2-thioxoimidazolidin-1-yl)phosphinate (IV) : Yield: 63%, m.p.:160-163°C, IR (KBr,cm^{-1}): 3065(C–H $_{aro,str.}$),1680cm-1 (C=$O_{str.}$), 1489cm-1 (C=C $_{aro,str.}$), 1150 cm-1 (C=S $_{aro,str.}$), 1300 (P=O), 965 (P-O), 1245 (O-C $_{aro,str}$), 750 (P-N), 768 cm-1 (C-Cl). $^{1}H_{str.}$NMR (DMSO-d_6 ,δ) : 6.89-8.24 (m ,12H, Ar-H), 4.39 (s, 4H, CH_2). ^{31}P NMR (DMSO-d_6 ,δ): -18.63 . Anal. Found (Calcd)% for $C_{24}H_{16}O_8N_6S_2PCl$: C, 44.2(44.4); H, 2.2(2.4); N, 12.6(12.9); S, 9.7(9.9); Cl, 5.0(5.2).

Chlorophenyl bis(3-(2,4-dichlorophenyl)-4-oxo-2-thioxoimidazolidin-1-yl)phosphinate (V): Yield: 64%, m.p.: 126-128°C, IR (KBr,cm^{-1}): 3069 (C–H $_{aro,str.}$),1675cm-1 (C=$O_{str.}$), 1483cm-1 (C=C $_{aro,str.}$), 1142 cm-1 (C=S $_{aro,str.}$), 1284 (P=O), 963 (P-O), 1242 (O-C $_{aro,str}$), 733 (P-N), 778 cm-1 (4-C-Cl). $^{1}H_{str.}$NMR (DMSO-d_6 ,δ) : 6.89-8.01 (m ,10H, Ar-H), 4.39 (s, 4H, CH_2). ^{31}P NMR (DMSO-d_6 ,δ): -18.63 . Anal. Found (Calcd)% for $C_{24}H_{14}O_4N_4S_2PCl_5$: C, 41.2(41.4); H, 1.7(2.0); N, 7.8(8.0); S, 9.0(9.2);Cl, 25.4(25.5).

Clorophenyl bis(3-(2,5-dichlorophenyl)-4-oxo-2-thioxoimidazolidin-1-yl)phosphinate (VI): Yield: 65%, m.p.: 131-133°C, IR (KBr,cm^{-1}): 3070 (C–H $_{aro,str.}$),1676cm-1 (C=$O_{str.}$), 1485cm-1 (C=C $_{aro,str.}$), 1140 cm-1 (C=S $_{aro,str.}$), 1290 (P=O), 966 (P-O), 1245 (O-C $_{aro,str}$), 738 (P-N), 778 cm-1 (4-C-Cl). $^{1}H_{str.}$NMR (DMSO-d_6 ,δ) : 6.80-7.80 (m ,10H, Ar-H), 4.39 (s, 4H, CH_2). ^{31}P NMR (DMSO-d_6 ,δ): -18.63 . Anal. Found (Calcd)% for $C_{24}H_{14}O_4N_4S_2PCl_5$: C, 41.2(41.4); H, 1.7(2.0); N, 7.8(8.0); S, 9.0(9.2);Cl, 25.4(25.5).

Chlorophenyl bis(3-(2-methoxy,4-nitrophenyl)-4-oxo-2-thioxoimidazolidin-1-yl)phosphinate (VII): Yield: 63%, m.p.: 164-166°C, IR (KBr,cm^{-1}): 3070 (C–H $_{aro,str.}$),1676cm-1 (C=$O_{str.}$), 1485cm-1 (C=C $_{aro,str.}$), 1140 cm-1 (C=S $_{aro,str.}$), 1290 (P=O), 966 (P-O), 1245 (O-C $_{aro,str}$), 738 (P-N), 772 cm-1 (4-C-Cl). $^{1}H_{str.}$NMR (DMSO-d_6 ,δ) : 6.89-7.96 (m ,10H, Ar-H), 4.39 (s, 4H, CH_2), 3.83(s, 6H, CH_3O). ^{31}P NMR (DMSO-d_6 ,δ): -18.63 . Anal. Found (Calcd)% for $C_{26}H_{20}O_{10}N_6S_2PCl$: C, 45.8(46.1); H, 2.7(2.9); N, 12.1(12.4); S, 9.2(9.5);Cl, 5.0(5.2).

Chlorophenyl bis(3-(4-methoxy,2-nitrophenyl)-4-oxo-2-thioxoimidazolidin-1-yl)phosphinate (VIII): Yield: 64%, m.p.: 148-150°C, IR (KBr,cm^{-1}): 3070 (C–H $_{aro,str.}$),1676cm-1 (C=$O_{str.}$), 1485cm-1 (C=C $_{aro,str.}$), 1140 cm-1 (C=S $_{aro,str.}$), 1290 (P=O), 966 (P-O), 1245 (O-C $_{aro,str}$), 738 (P-N), 778 cm-1 (4-C-Cl). $^{1}H_{str.}$NMR (DMSO-d_6 ,δ) : 6.89-7.96 (m ,10H, Ar-H), 4.39 (s, 4H, CH_2), 3.83(s, 6H, CH_3O). ^{31}P NMR (DMSO-d_6 ,δ): -18.63 . Anal. Found (Calcd)% for $C_{26}H_{20}O_{10}N_6S_2PCl$: C, 45.9(46.1); H, 2.7(2.9); N, 12.2(12.4); S, 9.2(9.5);Cl, 5.0(5.2).

Chlorophenyl bis(3-(2-methoxy,4-nitrophenyl)-4-oxo-2-thioxoimidazolidin-1-yl)phosphinate (IX): Yield: 660%, m.p.: 158-160°C, IR (KBr,cm^{-1}): 3078 (C–H $_{aro,str.}$),1680cm-1 (C=$O_{str.}$), 1490cm-1 (C=C $_{aro,str.}$), 1146 cm-1 (C=S $_{aro,str.}$), 1296 (P=O), 964 (P-O), 1248 (O-C $_{aro,str}$), 742 (P-N), 767 cm-1 (4-C-Cl),NO_2, OCH_3 $^{1}H_{str.}$NMR (DMSO-d_6 ,δ) : 6.89-7.96 (m ,10H, Ar-H), 4.39 (s, 4H, CH_2), 3.83(s, 6H, CH_3O). ^{31}P NMR (DMSO-d_6 ,δ): -18.63 . Anal. Found (Calcd)% for $C_{26}H_{20}O_{10}N_6S_2PCl$: C, 45.9(46.1); H, 2.7(2.9); N, 12.2(12.4); S, 9.2(9.5);Cl, 5.0(5.2).

Conclusions:-

A series of novel organophosphorus compounds were synthesized by the reactions of 4-Chlorophenyldichlorophosphate with 3-(substituted phenyl)-2-thioxoimidazolidin-4-one ligands with the aim to develop better antimicrobial agent. The results of biological tests make both thiohydantoin and phosphorus interesting lead molecules for further synthetic and biological evaluation. It can be concluded that this class of compound certainly hold great promise for discovering safer antimicrobial agents.

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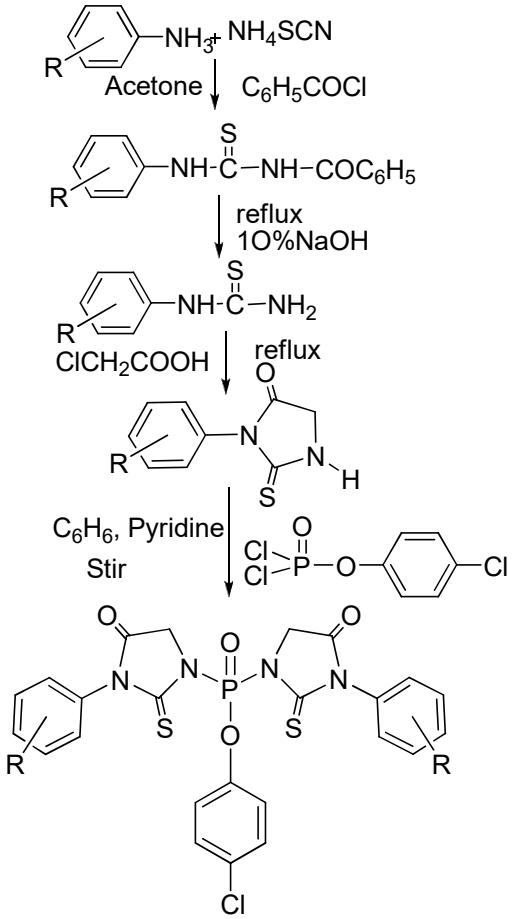

Scheme: Synthetic route of organophosphorus compounds containing substituted thiohydantoins

Table 1:- Reactions of 4-Chlorophenyl dichlorophosphate with 3-(substituted phenyl)-2-thioxoimidazolidin-4-one ligands

Comps.	Reactants Taken		Molar Ratio	Stirring Time (h)	Product
	$(ClC_6H_5O)POCl_2$ (mL)	Ligands (g)			
I	1.6	4.5(L1)	1:2	12	$C_{24}H_{16}O_4N_4S_2PCl_3$
II	1.6	5.4(L2)	1:2	14	$C_{24}H_{16}O_4N_4S_2PClBr_2$
III	1.6	4.2(L3)	1:2	14	$C_{24}H_{16}O_4N_4S_2PClF_2$
IV	1.6	4.7(L4)	1:2	10	$C_{24}H_{16}O_8N_6S_2PCl$
V	1.6	5.2(L5)	1:2	11	$C_{24}H_{14}O_4N_4S_2PCl_5$
VI	1.6	5.3(L6)	1:2	17	$C_{24}H_{14}O_4N_4S_2PCl_5$
VII	1.6	5.3(L1)	1:2	15	$C_{26}H_{20}O_{10}N_6S_2PCl$
VIII	1.6	5.4(L2)	1:2	18	$C_{26}H_{20}O_{10}N_6S_2PCl$
IX	1.6	4.4(L3)	1:2	12	$C_{28}H_{26}O_4N_4S_2PCl$

Where,
L1=3-(3-chlorophenyl)-2-thioxoimidazolidin-4-one,
L2=3-(4-bromophenyl)-2-thioxoimidazolidin-4-one,
L3= 3-(4-fluorophenyl)-2-thioxoimidazolidin-4-one ,
L4 = 3-(4-nitrophenyl)-2-thioxoimidazolidin-4-one
L5 = 3-(2,4-dichlorophenyl)-2-thioxoimidazolidin-4-one,
L6 = 3-(2,5-dichlorophenyl)-2-thioxoimidazolidin-4-one,
L7 = 3-(2-methoxy-4-nitrophenyl)-2-thioxoimidazolidin-4-one,
L8 = 3-(4-methoxy-2-nitrophenyl)-2-thioxoimidazolidin-4-one,
L9= 3-(3,4-dimethylphenyl)-2-thioxoimidazolidin-4-one.

Table 2:- Antibactirial screening data of organophosphorus compounds (zone of inhibition in mm).

Compounds	*Escherichia coli*			*Staphylococcus aureus*		
	250ppm	500ppm	1000ppm	250ppm	500ppm	1000ppm
I	7.6	9.3	11.3	10.6	12.6	14.3
II	7.0	8.6	10.0	7.3	8.6	11.6
III	6.3	8.0	10.0	7.3	8.6	11.6
IV	8.0	10.3	12.6	8.3	9.6	12.0
V	7.6	8.6	10.6	6.6	7.3	9.3
VI	7.3	8.3	10.0	7.3	8.6	10.6
VII	8.0	10.3	12.6	8.0	9.6	12.3
VIII	8.3	10.6	12.6	7.3	9.0	11.6
IX	10.3	11.6	13.3	12.6	13.6	15.3
Streptomycin	12.6	15.3	20.0	14.0	17.3	21.3

Table 3:- Antifungal screening data of organophosphorus compounds (zone of inhibition in mm).

Compounds	*Aspergillus niger*			*Aspergillus ochraceus*			*Fusarium oxyporum*		
	250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
I	7.3	9.6	11.3	9.6	11.3	13.6	9.3	12.0	14.0
II	12.3	14.6	18.0	8.0	9.6	12.0	13.6	16.0	19.3
III	10.6	12.6	14.3	6.6	8.3	10.0	12.6	13.6	17.0
IV	13.3	16.0	18.3	7.3	8.3	10.0	12.0	13.3	15.0
V	10.6	12.3	14.6	11.3	14.0	16.0	12.3	13.3	16.0
VI	8.6	11.3	13.0	10.0	13.3	15.3	9.3	11.6	14.3
VII	10.0	12.6	15.6	9.0	10.3	12.0	8.6	11.0	13.3
VIII	9.3	12.0	15.0	7.0	9.3	10.6	10.3	13.0	14.6
IX	14.6	17.3	19.6	8.6	10.6	12.6	13.0	15.0	16.3
Griseofulvin	15.3	18.0	20.6	13.6	15.6	17.0	15.0	17.6	20.3

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