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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW PHOSPHORUS HETEROCYCLES WITH EXOCYCLIC P–C/ P-O LINK

Kalpana Chaturvedi, Vinod Kumar Yadav & Smita Chaturvedi

Department of Chemistry, Agra College, Agra, 282002, India

E-mail – dr.kchat@rediffmail.com

Abstract

A series of biologically active organophosphorus compounds have been synthesized by the reactions of Phenylphosphonic dichloride / 4-Chlorophenyl dichlorophosphate with 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol, which in addition of being a nitrogen heterocyclic also contain –N=C-S- grouping responsible for biological activity. 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 anti microbial activity. They were found to possess significant anti-microbial activity.

***Keywords**: Organophosphorus, IR, NMR, anti-microbial activity.*

1. Introduction

Organophosphorus compounds are ubiquitous in nature and find applications in the field of agriculture, medicine and industry, (Breuer,1996, Prakasha, 1994). Some organophosphorus compounds have been described in the literature as inhibitors of bacterial (Alexakis,1991, Faraci, 1995) herbicides, insecticides, pesticides (Fest, 1982, Nivsarkar 2004), antifungal agents(Ali, 2000), anti-HIV (Mehellou, 2007), anti-cancer (Gulgan, 2005), antiviral andanti-inflammatory(Wittine, 2009). An important group of this class is phosphora-midate, which have been used in many reactions and synthesis of organic compounds. A number of research groups have become interested in organophosphorus heterocyclic compounds since they are finding extensive use as pesticides in agriculture, as stabilizers in polymers and as lubricant oil additives. A few recent studies (Sengupta,1998, 1998, 2002, 2003, Kevhan, 2017) 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. One of the useful properties of phosphorus compounds is their relatively low stability and rapid metabolic breakdown in plants, in animals, in organisms, in soil and in other components of the environment with the formation

of products that are safe for human beings and domestic animals. Another important feature of these compounds is the high selectivity of their action. 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. The present communication includes the reactions of Phenylphosphonic dichloride / 4-Chlorophenyldichlorophosphate with 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol., which in addition of being a nitrogen heterocyclic also contain –N=C-S- grouping responsible for biological activity. the structure of the ligands is given in Fig.1 and toxicological activity of these newly synthesized organophosphorus compounds against various important bacterial and fungal pathogens.

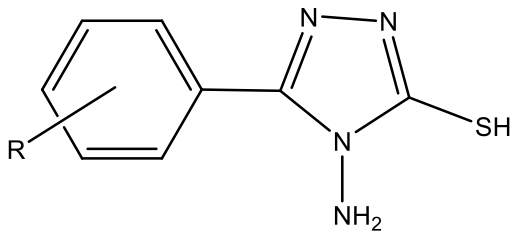


Fig.1- 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol

Where,

(I) R=H, (II) R=2-Br, (III) R=4-Cl, (IV) R=2,4-Cl_2,

(V) R=H, (VI) R=2-Br, (VII) R=4-Cl, (VIII) R=2,4-Cl_2,

2.Materials and Methods

The reactions of Phenylphosphonic dichloride / 4-Chlorophenyldichlorophosphate were carried out under inert atmosphere and anhydrous conditions. Special precautions were taken to exclude moisture from the apparatus and the starting materials (Phenylphosphonic dichloride / 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 (Sengupta,1998). Phenylphosphonic dichloride / 4-Chlorophenyl dichlorophosphate were procured from Aldrich Chemical Company, Inc. USA and were used without further purification. Substituted benzoic acid hydrazides were synthesized according to method described in the literature (Efimovsky, 1954, Teriunoby,

1969). The details of analysis and physical measurements were the same as reported earlier (Schrader, 1995).

3. Experimental

3.1 General procedure for the synthesis of 4-amino-5-(substituted phenyl)-4H-1, 2, 4-triazole-3-thiol

4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol were prepared by the reported method (George, 1969). To a solution containing 400 mL of methanol and (0.1 mol, 5.6 g) of potassium hydroxide (dissolved in 15 mL of water), (0.1 mol) of the appropriate hydrazide was added, (0.1 mol ,6.6 mL) of carbon disulfide was added with continuous shaking. After 2 hours,a solid mass of corresponding potassium aroyl dithiocarbazates was obtained,which was refluxed with excess of hydrazine hydrate (0.125 mol) for about 4 hours. The reaction mixture was cooled, poured in cold water and neutralized with dilute hydrochloric acid. The product, thus obtained, was filtered off, dried and re-crystallized from methanol. The IR spectra showed a weak S-H stretching absorption at 2480-2550 cm^{-1}.

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

The organphosphorus compounds were prepared by mixing Phenylphosphonic dichloride/4-Chlorophenyl dichlorophosphate (1 mol) and the appropriate ligand 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol (1 mol) in benzene (30 mL) in presence of pyridine (1 mol) with continuous stirring. Stirring was continued at room temperature over a period of 7-12 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.

Table1: Reactions of Phenylphosphonicdichloride /4-chlorophenyldichlorophosphate with substituted amino mercapto triazoles:

Reactants Taken $(C_6H_5)POCl_2$/ $(4\text{-}ClC_6H_4O)POCl_2$ (ml.)	4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol(g.)	Molar Ratio	Stirring Time (hrs.)	Product	Colour	Yield (%)
1.4	$C_8H_8N_4S$	1:1	8	$C_{14}H_{11}ON_4SP$	Light Yellow	72
1.4	$C_8H_7N_4SBr$	1:1	7	$C_{14}H_{10}ON_4SPBr$	Yellow	54
1.4	$C_8H_7N_4SCl$	1:1	12	$C_{14}H_{10}ON_4SPCl$	Light Yellow	70

1.4	$C_8H_6N_4SCl_2$	1:1	10	$C_{14}H_9ON_4SPCl_2$	White	64
1.6	$C_8H_8N_4S$	1:1	6	$C_{14}H_{10}O_2N_4SPCl$	Cream	68
1.6	$C_8H_7N_4SBr$	1:1	8	$C_{14}H_9O_2N_4SPBrCl$	Brown	61
1.6	$C_8H_7N_4SCl$	1:1	9	$C_{14}H_9O_2N_4SPCl_2$	Light Yellow	58
1.6	$C_8H_6N_4SCl_2$	1:1	10	$C_{14}H_8O_2N_4SPCl_3$	Creamy White	69

3.2.1 Organophosphorus derivative containing 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (I) : Mp190-192°C, IR (KBr,cm^{-1}): 3086 (P-N-H), 2941 (C–H $_{aro,str.}$), 760 (C-H $_{aro.oop.}$), 1610 (S-C=N) , 690 (P-S-C), 1569 (N-N=C), 1280 (P=O), 969 (P-$C_{aro.}$). ^{1}H NMR (DMSO-d_6 ,δ) : 7.41-7.66 (m ,10H, Ar-H), 7.8 (s, 1H, NH) . ^{31}P NMR (DMSO-d_6 ,δ): 12.63 . Anal. Found (Calcd)% for $C_{14}H_{11}ON_4SP$: C, 53.2(53.4); H, 3.3(3.5); N, 17.6(17.8); S, 10.0(10.2).

3.2.2 Organophosphorus derivative containing 4-amino-5-(2-bromophenyl)-4H-1,2,4-triazole-3-thiol (II) : Mp107-109°C, IR (KBr,cm^{-1}): 3081 (P-N-H), 2939 (C–H $_{aro,str.}$), 742 (C-H $_{aro.oop.}$), 1623 (S-C=N) , 698 (P-C-S), 1532 (N-N=C), 1174 (P=O), 947 (P-$C_{aro.}$), 1043 (C-Br_{str}).. ^{1}H NMR (DMSO-d_6 ,δ) : 7.30-7.77 (m ,9H, Ar-H). 8.8 (s, 1H, NH) ^{31}P NMR (DMSO-d_6 ,δ): 12.65 . Anal. Found (Calcd)% for $C_{14}H_{10}ON_4SPBr$: C, 42.5(42.7); H, 2.3(2.5); N, 14.0(14.2); S, 14.0(14.2); Br, 20.1(20.3).

3.2.3 Organophosphorus derivative containing 4-amino-5-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol (III) : Mp 230-233°C, IR (KBr,cm^{-1}): 3292 (P-N-H), 2925 (C–H $_{aro,str.}$), 756 (C-H $_{aro.oop.}$), 1648 (S-C=N) , 749 (P-S-C), 1604 (N-N=C), 1329 (P=O), 935 (P-$C_{aro.}$), 1092 (C-Cl_{str}). ^{1}H NMR (DMSO-d_6 ,δ) : 7.45-7.91 (m ,7H, Ar-H), 8.12 (d, 2H, Ar-H), 9.0 (s, 1H, NH). ^{31}P NMR (DMSO-d_6 ,δ): 12.7 . Anal. Found (Calcd)% for $C_{14}H_{10}ON_4SPCl$: C, 48.0(48.1); H, 2.6(2.8); N, 15.8(16.0); S, 9.0(9.2); Cl, 9.9(10.0).

3.2.4 Organophosphorus derivative containing 4-amino-5-(2,4-dichlorophenyl)-4H-1,2,4-triazole-3-thiol (IV) : Mp 130-132°C, IR (KBr,cm^{-1}): 3288 (P-N-H), 3080 (C–H $_{aro,str.}$), 781 (C-H $_{aro.oop.}$), 1598 (S-C=N) , 684 (P-S-C), 1556 (N-N=C), 1282 (P=O), 969 (P-$C_{aro.}$), 1092 (C-Cl_{str}).. ^{1}H NMR (DMSO-d_6 ,δ) : 7.6-7.86 (m ,8H, Ar-H), 7.9 (s, 1H, NH). ^{31}P NMR (DMSO-d_6 ,δ): 12.68 . Anal. Found (Calcd)% for $C_{14}H_9ON_4SPCl_2$: C, 43.6(43.8); H, 2.1(2.3); N, 14.4(14.6); S, 8.1(8.2); Cl, 18.0(18.2).

3.2.5 Organophosphorus derivative containing 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (V) : Mp 163-165°C, IR (KBr,cm^{-1}): 3083 (P-N-H), 2939 (C–H $_{aro,str.}$), 760 (C-H

$_{aro.oop.}$), 1610 (S-C=N) , 692 (P-S-C), 1569 (N-N=C), 1280 (P=O), 970 (P-O), 1238 (O-$C_{aro.}$), 1021 (C-Cl_{str}).. ^{1}H NMR (DMSO-d_6 ,δ) : 6.89-7.66 (m ,9H, Ar-H), 7.89 (s, 1H, NH). ^{31}P NMR (DMSO-d_6 ,δ): -18.28 . Anal. Found (Calcd)% for $C_{14}H_{10}N_4PSCl$: C, 45.8(50.0); H, 2.5(2.7); N, 15.1(15.3); S, 8.6(8.8); Cl, 9.7(9.9).

3.2.6 Organophosphorus derivative containing 4-amino-5-(2-bromophenyl)-4H-1,2,4-triazole-3-thiol (VI) : Mp109-111°C, IR (KBr,cm^{-1}): 3313 (P-N-H), 2937 (C–H $_{aro,str.}$), 737 (C-H $_{aro.oop.}$), 1622 (S-C=N) , 696 (P-S-C), 1520 (N-N=C), 1227 (P=O), 949 (P-O), 1177 (O-$C_{aro.}$), 1030 (C-Br_{str}), 1090 (C-Cl_{str}). ^{1}H NMR (DMSO-d_6 ,δ) : 6.89-7.68 (m ,8H, Ar-H), 8.9 (s, 1H, NH). ^{31}P NMR (DMSO-d_6 ,δ): -18.24 . Anal. Found (Calcd)% for $C_{14}H_{10}ON_4SPBr$: C, 42.5(42.7); H, 2.3(2.5); N, 14.0(14.2); S, 14.0(14.2); Br, 20.1(20.3).

3.2.7 Organophosphorus derivative containing 4-amino-5-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol (VII) : Mp 150-152°C, IR (KBr,cm^{-1}): 3347 (P-N-H), 2925 (C–H $_{aro,str.}$), 756 (C-H $_{aro.oop.}$), 1678 (S-C=N) , 756 (P-S-C), 1597 (N-N=C), 1211 (P=O), 960 (P-O), 1142 (O-$C_{aro.}$), 1094 (C-Cl_{str}). ^{1}H NMR (DMSO-d_6 ,δ) : 7.11-7.56 (m ,8H, Ar-H), 7.9 (s, 1H, NH) . ^{31}P NMR (DMSO-d_6 ,δ): -18.21 . Anal. Found (Calcd)% for $C_{14}H_9O_2N_4SPCl_2$: C, 41.8(42.0); H, 2.0(2.2); N, 13.8(14.0); S, 7.8(8.0); Cl, 17.5(17.7).

3.2.8 Organophosphorus derivative containing 4-amino-5-(2,4-dichlorophenyl)-4H-1,2,4-triazole-3-thiol (VIII) : Mp 105-107°C, IR (KBr,cm^{-1}): 3286 (P-N-H), 3078 (C–H $_{aro,str.}$), 757 (C-H $_{aro.oop.}$), 1676 (S-C=N) , 677 (P-S-C), 1596 (N-N=C), 1230 (P=O), 946 (P-O), 1155 (O-$C_{aro.}$), 1095 (C-Cl_{str}). ^{1}H NMR (DMSO-d_6 ,δ) : 6.9-7.6 (m, 7H, Ar-H), 7.8 (s, 1H, NH). ^{31}P NMR (DMSO-d_6 ,δ): 18.26. Anal. Found (Calcd)% for $C_{14}H_8O_2N_4SPCl_3$: C, 38.5(38.7); H, 1.6(1.7); N, 12.7(12.9); S, 7.2(7.4); Cl, 24.3(24.5).

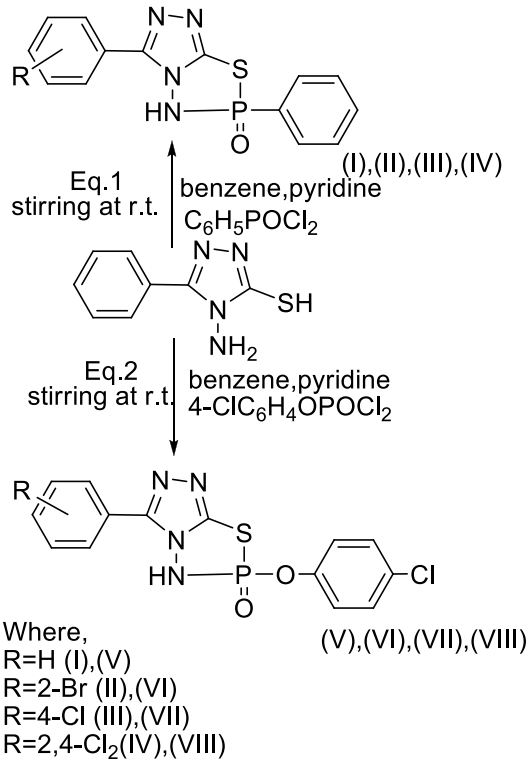
3.3 Antimicrobial Activity

Antimicrobial test was performed on two bacteria (*Staphylococcus aureus* and *Escherichia coli*) and three fungi (*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. 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 up to 10mL with 9.9mL of sterile deionised 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 all newly synthesized organophosphorus compounds were evaluated in vitro against Gram positive bacteria- *Staphylococcus aureus* and Gram negative bacteria- *Escherichia coli* (Table 2).

4. **Results and Discussion**

Reactions of Phenylphosphonic dichloride / 4-Chlorophenyl dichlorophosphate with 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol ligands have been carried out in benzene in the presence of pyridine and a variety of organophosphorus derivatives have been isolated according to Eqs.(1), and (2)**.** The methods used for the preparation and isolation of these compounds gave materials of good purity as supported by their analyses and TLC. 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 whitish cream to brown in colour. The compounds melt in the temperature range of 107-230°C.



4.1 Infrared spectra

The assignments of infrared spectral bands of the ligands and the compounds are based on earlier studies on similar ligands (Fobretti, 1983, Pandey,1984, Bahel,S.C.,1982, Singh,1969, Dwivedi, 1972, Goel,1989). The infrared spectra of mercapto amino triazoles (in solution) show one weak band at ca.2480-2550 cm^{-1} due to ν(S-H). However, in the spectra of organophosphorus derivatives, this band disappear which confirm the coordination through sulphur after deprotonation. This is further supported by appearance of band at ca.677-756 cm^{-1} assignable (Nakamoto, 1970) to ν(P-S-C).

The infrared spectra of substituted mercapto amino triazoles show bands at ca. 3400-3250 cm^{-1} and at ca.2480-2550 cm^{-1} due to the –NH and –SH group vibrations respectively. However, in the spectra of all organophosphorus derivatives, these bands disappear which confirm the coordination through nitrogen of amino group and sulphur of thiol group after deprotonation. This is further supported by appearance of band at ca. 677-756 cm^{-1} and 3083-3343 cm^{-1} due to ν(P-S-C) and ν(P-N-H) respectively in organophosphorus derivatives.

The bands observed around 1598-1678 cm^{-1} and 1520-1604 cm^{-1} in the spectra of triazoles may be assigned (Fobretti, 1983, Singh, 1969, Pandey, 1984) to ν(S-C=N) and ν(N-N=C) ring vibrations, respectively. All these bands persist in organophosphorus derivatives indicating the non- coordination of azomethine nitrogens.

4.2 Nuclear Magnetic Resonance Spectra

The ^{1}H NMR spectra were recorded on a Bruker Avance III, 400MHz spectrometer operating at 400 MHz to ^{1}H and 161.9 MHz for ^{31}P NMR using DMSO-d_6 as solvent. Of course, the protons of R groups in the mercapto amino triazoles 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 6.89-7.77. The signals due to –SH **and** $–NH_2$ protons appears at *ca*. δ 13.05 (Reddy, 2004) and *ca*. δ 5.1-5.70 (Lakshman, 2010) respectively in the spectra of all mercapto amino triazoles ligands which disappears in their corresponding organophosphorus derivatives indicating the deprotonation of S–H proton and $–NH_2$ proton and formation of P-S and P-NH bonds.

^{31}P NMR chemical shifts of the compounds (I, II, III, IV) appeared in the region *ca*. δ 12.63 ppm whereas in the compounds (V, VI, VII, VIII) signals appear in the region *ca*. δ -18.21ppm.

4.3 Antimicrobial Activity

Antimicrobial activities of all newly synthesized organophosphorus compounds were evaluated in vitro against Gram positive bacteria- *Staphylococcus aureus* and Gram negative bacteria- *Escherichia coli* (Table 2). The majority of the compounds (I-VIII) exhibited moderate to good activity 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-VIII) exhibited moderate to excellent antifungal activity when compared with the Griseofulvin in reference.

Table 2: Antibacterial activities of organophosphorus compounds[zones of Inhibition (in mm)]:

Compounds	Escherichia coli			Staphylococcus aureus		
	250ppm	500ppm	1000ppm	250ppm	500ppm	1000ppm
$C_{14}H_{11}ON_4SP$(I)	7.0	7.3	8.3	7.0	8.0	10.6
$C_{14}H_{10}ON_4SPBr$(II)	7.3	7.3	8.6	13.0	15.3	16.6
$C_{14}H_{10}ON_4SPCl$(III)	7.6	8.6	10.6	6.6	8.0	10.6
$C_{14}H_9ON_4SPCl$(IV)	5.6	6.6	8.0	6.3	8.0	10.3
$C_{14}H_{10}O_2N_4SPCl$(V)	6.3	6.6	7.3	7.0	8.3	10.6
$C_{14}H_9O_2N_4SPBrCl$(VI)	5.3	6.6	7.3	7.6	9.6	11.6
$C_{14}H_9O_2N_4SPCl_2$9VII)	6.3	7.0	8.0	6.3	7.0	9.6
$C_{14}H_8O_2N_4SPCl_3$(VIII)	8.6	10.0	11.3	7.0	7.6	10.0
Streptomycin	12.6	15.3	20.0	14.0	17.3	21.3

Table 3: Antifungal activities of organophosphorus derivatives [zones of inhibition (in mm)]:

Compounds	Aspergillus niger			Aspergillus ochraceus			Fusarium oxyporum		
	250ppm	500ppm	1000ppm	250ppm	500ppm	1000ppm	250ppm	500ppm	1000ppm
$C_{14}H_{11}ON_4SP$(I)	10.0	11.3	14.0	7.3	9.0	10.0	10.6	15.3	18.6
$C_{14}H_{10}ON_4SPBr$(II)	9.3	10.6	11.6	6.6	8.0	9.6	8.0	11.6	12.3
$C_{14}H_{10}ON_4SPCl$(III)	10.6	12.6	16.6	8.6	10.3	12.6	6.6	8.6	13.6
$C_{14}H_9ON_4SPCl_2$(IV)	15.0	18.0	20.0	12.3	15.6	17.0	15.0	17.6	20.3
$C_{14}H_{10}O_2N_4SPCl$(V)	11.6	13.3	17.0	7.6	9.6	11.3	14.3	17.0	20.0
$C_{14}H_9O_2N_4SPBrCl$(VI)	5.3	8.3	8.6	7.0	8.6	10.6	7.3	9.0	10.3
$C_{14}H_9O_2N_4SPCl_2$(VII)	9.3	11.0	16.0	7.3	9.3	10.3	5.0	7.6	10.3
$C_{14}H_8O_2N_4SPCl_3$(VIII)	10.6	13.0	15.6	9.6	11.3	13.0	10.3	13.0	17.0
Griseofulvin	15.3	18.0	20.6	13.6	15.6	17.0	15.0	17.6	20.3

5. Conclusion

An elegant synthesis of novel organophosphorus compounds containing 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol with high yields is accomplished and their antimicrobial activity was evaluated. They exhibited promising antimicrobial activity.

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