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Synthesis of novel urea and thiourea derivatives of diphenylphosphoramidate and their antimicrobial activity

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ABSTRACT

A series of novel urea/thiourea derivatives of diphenylphosphoramidate have been synthesized in two steps. In the first step, 4-aminoaniline/4, 4'diaminodiphenylsulfone (Dapsone) was reacted with diphenyl chlorophosphate in the presence of triethylamine (TEA) to get intermediates. Intermediates further treated with various substituted aromatic isocyanates/ isothiocyanates in the presence of TEA to obtain the title compounds. The structures of newly synthesized compounds were confirmed by IR, NMR (^{1}H, ^{13}C, and ^{31}P), mass and elemental analysis. The title compounds showed good antibacterial activities against Gram-positive and Gram-negative bacteria.

Key words: Urea /thiourea derivatives, Dapsone, 4-Aminoaniline, Antimicrobial activity.

INTRODUCTION

Bacterial infections have increased dramatically in recent years. Bacteria have been the cause of the most deadly diseases and widespread epidemics in human civilization. Infectious diseases caused by bacteria and fungi remain as a major world health problem due to rapid development of resistance to the existing antimicrobial drugs (antibacterial and antifungal). In other words, the increasing use and misuse of the existing antimicrobial drugs have resulted in the development of resistant pathogens. In particular, the emergence of multi-drug resistant gram-positive and gram-negative bacteria has caused life-threatening infectious diseases in many countries around the world [1]. In addition, systemic and dermal fungal infections have significantly increased, specifically in individuals with suppressed immune systems such as AIDS patients [2]. Urea and thiourea derivatives show a broad spectrum of biological activities such as antibacterial, antifungal, antiviral, anticancer, anticonvulsant, analgesic and HDL-elevating [3-8].

Urea was the first synthesized organic compound, it was artificially synthesized in synthetic laboratory by Wohler in 1828. Urea is synthesized in the body of many organisms as part of the urea cycle. Urea production occurs in the liver and found in the urine of mammals, dissolved in blood, plants, birds, yeast and many microorganisms. Aromatic urea derivatives such as N-phenyl-N-(2-chloroethyl) urea and hetero cyclic urea derivatives show good anti-cancer activity due to their good inhibitory activity against receptor tyrosine kinases(RTKs) [9]. Urea has many applications in agriculture, industries, laboratories, automobiles, fertilizers, medicines, urinetherapy and enzyme urease. Diphenyl urea derivatives are inhibitors of transketolase [10]. Urea derivatives show good biological activities such as antimicrobial, anticancer [11] and anaplastic lymphoma kinase (ALK) inhibitors [12].

Numerous compounds containing thiourea group are selective ligands for 5-HT family receptors, including 5-$HT_{2A,}$ 5-HT_{2B} and 5-HT_{2C} [13-14]. Structural studies of active thiourea derivatives have shown that these compounds contain a central hydrophilic part and two hydrophobic moieties forming a butterfly-like conformation [15]. This

conformation is a part of structure of NNRTIs (Non-nucleoside reverse transcriptase inhibitors) and anti-HIV agents [16]. Thiourea derivatives have been found to possess many promising biological activities [17], many of them being used as herbicides [18], insecticides [19], plant-growth regulators [20], antibacterial [21], antifungal [22], tuberculostatic [23], antitumor [24], anticonvulsant [25] and antiviral properties[26].

Compounds that have phosphoramidate derivatives containing P-N function have a wide range of biological activities. The nitrogen atom source could be an external additive like guanidine and urea derivatives [27] or direct linkage of nitrogen to the phosphorous atom in the case of phosphoramidates [28]. Study and development of newer phosphoramidate derivatives are more important due to possible phosphorous-nitrogen (P-N) synergism phenomenon. Phosphoramidate is a phosphate that has two of its OR groups and –NR group. An example of a natural phosphoramidate is phosphocreatine, it used as cardioprotective agent and cyclophosphoramide is an anticancer drug. Most of the phosphoramidate derivatives have a range of biological activities such as anti-HIV and anti-viral (Reverse-Transcriptase (RT), DNA polymerase and protease) inhibiting properties [29].

Owing to the biological activities of urea, thiourea and phosphoramidates, we have designed and synthesized biologically active compounds through the combination of different pharmacophores in one structure.

MATERIALS AND METHODS

Experimental

Chemistry

All chemicals were purchased from Sigma Aldrich and Merck, and used as such without further purification. Solvents used for spectroscopic and other physical studies were reagent grade and were further purified by literature methods [30]. Melting points were determined using a capillary thermometer by GUNA Digital Melting Point Apparatus and are uncorrected. IR spectra were recorded as KBr pellets on SHIMADZU FT-IR 8400S spectrophotometer. ^{1}H & ^{13}C NMR spectra were recorded in DMSO-d_6 on Bruker AVANCE-**300** MHz spectrometer operating at 300 MHz for ^{1}H NMR, 75 MHz for ^{13}C NMR and 121 MHz for ^{31}P NMR. The ^{1}H, ^{13}C and ^{31}P NMR chemical shifts were expressed in ppm, ^{1}H and ^{13}C NMR spectra referenced to tetramethylsilane and ^{31}P NMR spectra referenced to 85% H_3PO_4. E.S.I mass spectra were recorded on a MLP2103 mass spectrometer. Elemental analysis was performed on Thermo Finnigan FLASH EA 1112 instrument.

General procedure for synthesis of title compounds 4 a-e and 7a-e.

4,4'-Sulfonyldianiline(**1**)/4-aminoaniline(**5**) (0.001mol) was taken in 50 mL round bottom flask in presence of triethylamine (0.001mol) and 10 mL of dry THF and diphenylchlorophosphate (**2**) (0.001 mol) in 10 mL of dry THF was added drop wise through a dropping funnel at 10-15 ^{0}C. After completion of the addition, the temperature was slowly raised to 40-45 ^{0}C continued for 3-5 h to form the product. The progress of the reaction was monitored by TLC using ethyl acetate and hexane (3:2). The triethylamine hydrochloride was removed from reaction mixture by filtration to get the diphenylphosphoramidate intermediate (**3**/**6**). Various aromatic substituted isocyanates/ thioisocyanates (0.001mol) were added to the **3/6** in presence of triethylamine, after addition of isocyanates/thioisocyanates, the temperature was slowly raised to 60-65 ^{0}C and stirred for 2-4 h to obtain urea/thiourea derivatives of diphenylphosphoramidate(**4 a-e / 7a-e**). The progress of the reaction was monitored by TLC using ethylacetate and hexane (3:2). The solvent was removed from reaction mixture in a rotaevaporator to get the crude product, it was purified by column chromatography to obtain the title compounds **4 a-e** and **7 a-e** with high yields (65-75%). The synthesis of title compounds is presented in **Scheme1**.

Diphenyl 4-(4-(3-phenylthioureido) phenylsulfonyl) phenylphosphoramidate (4a).

Colourless solid, yield 68%, M.wt:615, m.p 150-154 $^{\circ}$C, IR(KBr), (ν_{max}, cm^{-1}), 3470(N-H_{str}), 1593(N-H_{bend}), 1447 (C=S), 1405(C-N), 1260(P=O), 1183(P-O-$C_{aromatic}$), 960(P-N); ^{1}HNMR (DMSO-d_6), δ ppm, 9.75(s, 2H, NH), 9.55(s, 1H, Ar-NH), 7.80-6.80 (m, 23H, Ar-H); ^{13}CNMR (DMSO-d_6), δ (ppm), 184.4(C-13^{1}), 153.3(C-1), 148.9(C-1^{1}), 148.4(C-10^{1}), 143.4(C-1^{11}), 142(C-7^{1}), 136(C-4^{1}), 135(C-3&C-5), 134(C-3^{1}&C-5^{1}), 133.4(C-3^{11}&C-5^{11}), 132.8(C-8^{1}&C-12^{1}), 132.4 (C-4^{11}), 130.2(C-2^{11}&C-6^{11}), 129.1(C-9^{1}&C-11^{1}), 127.2(C-4), 120(C-2&C-6), 117.3(C-2^{1}&C-6^{1}); ^{31}PNMR (DMSO-d_6), 10.0 ppm, E.S.I. mass, m/z, (%), 615 ($M^{+\cdot}$)(100%), 384(30%); Ana.cal.for C31H26N3O5PS2; C, 60.48; H, 4.26; N, 6.83; Found: C, 60.36; H, 4.21; N, 6.75.

Diphenyl 4-(4(3(4-chlorophenyl)thioureido)phenylsulfonyl)phenylphosphoramidate(4b).

Browncolour solid, yield 71%, M.wt:650, m.p238-240 ^{0}C, IR(KBr), (ν_{max} ,cm^{-1}), 3477(N-H_{str}), 1592(N-H_{bend}), 1489(C=S), 1398(C-N), 1280(P=O), 1165(P-O-$C_{aromatic}$), 969(P-N); ^{1}H NMR (DMSO-d_6), δ (ppm), 10.8(s, 2H, Ar-NH), 8.8(s, 1H, Ar-NH), 7.1-7.8(m, 22H, Ar-H); ^{13}C NMR (DMSO-d_6), δ (ppm), 184.2(C-13^{1}), 153.0(C-1), 148.4(C-1^{1}), 148.2(C-10^{1}), 141.5(C-7^{1}), 141(C-1^{11}), 136.4 (C-4^{11}), 136(C-4^{1}), 135.2(C-2^{11}&C-6^{11}), 135(C-3&C-5),

134(C-3^1&C-5^1), 133.4(C-3^{11}&C-5^{11}), 132.8(C-8^1&C-12^1), 128.5(C-9^1&C-11^1), 127(C-4), 120.1(C-2&C-6), 117.3(C-2^1&C-6^1); ^{31}P NMR, (DMSO-d6), 10.15 ppm; E.S.I. mass, m/z, (%), 649 ($M^{+\cdot}$) (100), 651(M+2)(33%); Ana.cal.for $C_{31}H_{25}ClN_3O_5PS_2$; C, 57.27; H, 3.88; N, 6.46. Found: C, 57.20; H, 3.82; N, 6.40:

Diphenyl 4-(4-(3-(4-chlorophenyl)ureido)phenylsulfonyl)phenylphosphoramidate(4c)
Pale orange solid, yield 69%, M.wt:634, m.p 138-140 ^{0}C, IR(KBr), (ν_{max} ,cm^{-1}), 3474(N-H_{str}), 1697(C=O), 1592(N-H_{bend}), 1400(C-N), 1280(P=O), 1183(P-O-$C_{aromatic}$), 950(P-N); ^{1}H NMR(DMSO-d_6), δ(ppm), 8.80(s, 2H, Ar-NH),8.50(s, 1H, Ar-NH), 6.80-7.70(m, 22H, Ar-H); ^{13}C NMR(DMSO-d_6), δ(ppm), 153(C-13^1), 152.8(C-1), 148.6(C-1^1), 148.2(C-10^1), 136.0(C-1^{11}), 142(C-7^1), 135.8(C-4^1), 135(C-3&C-5), 134(C-3^1&C-5^1), 133.3(C-3^{11}&C-5^{11}), 132.6(C-8^1&C-12^1), 132.4 (C-4^{11}), 130.2(C-2^{11}&C-6^{11}), 127.2(C-4), 123.7(C-9^1&C-11^1), 120(C-2&C-6), 117.3(C-2^1&C-6^1); ^{31}P NMR (DMSO-d_6), 10.45 ppm; E.S.I, mass m/z (%), 633[$M^{+\cdot}$](100%), 635(M+2)(33); Ana.cal.for $C_{31}H_{25}ClN_3O_6PS$; C, 58.72; H, 3.97; N, 6.63. Found: C, 58.86; H, 3.89; N, 6.58.

Diphenyl4-(4-(3-(2-nitrophenyl)ureido)phenylsulfonyl)phenylphosphoramidate(4d)
Yellow solid, yield 70%, M.wt:644, m.p 258-260 ^{0}C, IR (KBr), (ν_{max}, cm^{-1}), 3471(N-H_{str}), 1714(C=O), 1592(N-H_{bend}), 1403(C-N). 1280(P=O), 1184(P-O-$C_{aromatic}$), 955(P-N); ^{1}H NMR (DMSO-d_6), δ(ppm), 10.20(s,1H, NH), 10.10(s, 1H, NH), 9.8(s, 1H, NH), 8.5(d, 1H, *J=8.4 Hz*, Ar-H), 8.15(d, 1H, *J=8.2 Hz*, Ar-H), 7.1-7.85(m, 20H, Ar-H); ^{13}C NMR (DMSO-d_6), δ (ppm), 153.2(C-13^1), 153(C-1), 148.9(C-1^1), 148.4(C-10^1), 143.4(C-1^{11}), 142(C-7^1), 136.4(C-4^1), 136(C-5^{11}), 135(C-3&C-5), 134(C-3^1&C-5^1), 132.8(C-8^1&C-12^1), 130.4(C-3^{11}), 130.2 (C-4^{11}), 147.2(C-2^{11}), 123.4(C-6^{11}), 123.1(C-9^1&C-11^1), 127.2(C-4), 120(C-2&C-6), 117.3(C-2^1&C-6^1); ^{31}P NMR (DMSO-d_6) 11.50ppm; E.S.I. mass m/z(%), 644($M^{+\cdot}$)(20%), 458(100%); Ana.cal.for $C_{31}H_{25}ClN_4O_8$ PS; C, 57.76; H, 3.91; N, 8.69. Found: C, 57.70; H, 3.87; N, 8.65.

Diphenyl 4-(4-(3-(4-nitrophenyl)thioureido)phenylsulfonyl)phenylphosphoramidate(4e).
Yellow solid, yield 72%, M.wt:660, m.p 158-160 $^\circ$C, IR(KBr), (ν_{max}, cm^{-1}), 3481(N-H_{str}), 1593(N-H_{bend}), 1443(C=S), 1395(C-N), 1262(P=O), 1180(P-O-$C_{aromatic}$), 967(P-N); ^{1}H NMR (DMSO-d_6), δppm, 10.5(s, 2H, Ar-NH), 8.65(s, 1H, Ar-NH),7.95(d, 2H, *J=8.2 Hz*, Ar-H), 6.94-7.7(m, 20H, Ar-H); ^{13}C NMR (DMSO-d_6), δ (ppm) 184.2(C-13^1), 153.3(C-1), 148.9(C-1^1), 148.4(C-10^1), 149.4(C-1^{11}), 142(C-7^1), 136(C-4^1), 135(C-3&C-5), 134(C-3^1&C-5^1), 129.2(C-3^{11}&C-5^{11}), 132.8(C-8^1&C-12^1), 147.9 (C-4^{11}), 128.8(C-2^{11}&C-6^{11}), 129.1(C-9^1&C-11^1), 127.2(C-4), 120(C-2&C-6), 117.3(C-2^1&C-6^1); ^{31}P NMR (DMSO-d_6), 10.25 ppm; E.S.I. mass m/z(%), 660($M^{+\cdot}$)(20%), 306(100); Ana.cal.for $C_{31}H_{25}ClN_4O_7PS_2$; C, 56.36; H, 3.81; N,8.48. Found: C, 56.30; H, 3.76; N, 8.42.

Diphenyl 4-(3-phenylthioureido) phenylphosphoramidate(7a).
Yellow solid, yield 70%, M.wt:475, m.p 115-118^0 C, IR(KBr), (ν_{max}, cm^{-1}), 3477(N-H_{str}), 1590(N-H_{bend}), 1450(C=S), 1380(C-N), 1260(P=O), 1163(P-O-$C_{aromatic}$), 950(P-N); ^{1}H NMR(DMSO-d), δ(ppm), 10.2(s, 2H, Ar-NH), 8.8(s, 1H, Ar-NH), 6.9-7.5(m, 19H, Ar-H); ^{13}C NMR(DMSO-d_6), δ, 184.6(C-7^1), 150(C-1), 138.8(C-1^1), 138.5(C-1^{11}), 129.9(C-3&C-5), 128.6(C-4^1), 128.5(C-3^{11}&C-5^{11}), 128.2(C-4^{11}), 127(C-3^1&C-5^1), 126.1(C-2^{11}&C-6^{11}), 121(C-4), 119.8(C-2&C-6), 118.3(C-2^1&C-6^1); ^{31}P NMR (DMSO-$d_{6,}$) -7.71 ppm; E.S.I. mass m/z (%) 475.50 [$M^{+\cdot}$, 20%], 229 (100%), Ana.cal.for $C_{25}H_{22}N_3O_3PS$; C, 63.15; H, 4.66; N, 8.84; Found: C, 63.10; H, 4.61; N, 8.79.

Diphhenyl 4-(3-(2-nitrophenyl)ureido) phenylphosphoramidate(7b).
Orange red solid, yield 75%, M.wt:504, m.p 88-92 ^{0}C, IR(KBr), (ν_{max}, cm^{-1}), 3478 (N-H_{str}), 1732 (C=O), 1570 (N-H_{bend}), 1390 (C-N), 1280 (P=O), 1069(P-O-$C_{aromatic}$), 948 (P-N); ^{1}H-NMR (DMSO-d_6), δ (ppm), 10.15(s, 1H, Ar-NH), 9.80(s, 1H, Ar-NH), 8.70(s, 1H, Ar-NH), 8.50(d, 1H, Ar-H), 8.20(d, 1H, Ar-H), 7.2-7.8(m, 16H, Ar-NH); ^{13}C NMR (DMSO-d_6) δ (ppm), 152.7 (C-7^1), 150.1 (C-1), 142.1(C-2^{11}), 138.8(C-1^1), 131.5(C-1^{11}), 131(C-5^{11}), 129.9(C-3&C-5), 129.2(C-4^1), 125.2(C-4^{11}),125(C-3^{11}), 122(C-3^1&C-5^1), 121(C-4), 119.8(C-2&C-6), 118.3(C-2^1&C-6^1); 118.2(C-6^{11}), ^{31}P NMR (DMSO-d_6) δ (ppm), -10.20 ppm; E.S.I. mass m/z (%) 504 [$M^{+\cdot}$,25%], 413 (30%), 301 (100%), 288 (11%), 260 (13%),; Ana.cal.for $C_{25}H_{21}N_4O_6P$: C, 59.53; H, 4.20; N, 11.11; Found : C, 59.45; H, 4.26; N, 11.02.

Diphhenyl 4-(3-(3-chloro-4-fluorophenyl)ureido)phenylphosphomidate(7c).
`Light brown solid, yield 72%, M.wt:511, m.p 218-221^0 C, IR (KBr), (ν_{max} ,cm^{-1}), 3487(N-H_{str}), 1700(C=O), 1563(N-H_{bend}), 1400(C-N), 1260(P=O), 1175(P-O-$C_{aromatic}$), 945(P-N); ^{1}H NMR(DMSO-d_6), δ (ppm), 9.5(s, 1H, Ar-NH), 8.6(s, 1H, Ar-NH), 8.2(s, 1H, Ar-NH), 7.0-7.8(m, 17H, Ar-H);^{13}C NMR (DMSO-d_6), δ(ppm), 160(C-3^{11}), 152.2(C-7^1), 150.1(C-1), 138.2(C-1^1), 135.5(C-1^{11}), 130.5(C-5^{11}), 129.9(C-3&C-5), 129(C-4^1), 122.2(C-3^1&C-5^1), 121.6(C-6^{11}), 121(C-4), 119.8(C-2&C-6), 116.7(C-4^{11}), 116.3, (C-2^1&C-6^1), 111.3(C-2^{11}); ^{31}P NMR (DMSO-d_6) -12.08 ppm ; E.S.I. mass m/z (%), 511[$M^{+\cdot}$, 100%], 513(M+2, 32%); Ana.cal.for $C_{25}H_{20}ClFN_3O_4P$; Elemental Analysis: C, 58.66; H, 3.94; N, 8.21; Found : C, 58.60; H, 3.88; N, 8.15.

Diphhenyl 4-(3-(4-chlorophenyl)thioureido)phenylphosphomidate(7d).
Yellow solid, yield 70%, M.wt:510, m.p.147-150 ^{0}C, IR (KBr), (ν_{max} ,cm^{-1}), 3470(N-H$_{str}$), 1590(N-H$_{bend}$), 1488(C=S), 1400(C-N), 1284(P=O), 1164(P-O-C$_{aromatic}$), 950(P-N); ^{1}H NMR(DMSO-d$_6$), δ(ppm), 10.5(m, 2H, Ar-NH), 9.45(m, 1H, Ar-NH), 7.0-7.7(m, 18H, Ar-H); ^{13}C NMR(DMSO-d$_6$), δ(ppm), 185(C-7^1), 150(C-1), 138.6(C-1^1), 136.5(C-1^{11}), 133.5(C-4^{11}), 131.4(C-2^{11}&C-6^{11}), 129.8(C-3&C-5), 129(C-3^{11}&C-5^{11}), 128.5(C-4^1), 127(C-3^1&C-5^1), 121(C-4), 119.8(C-2&C-6), 118.3(C-2^1&C-6^1); ^{31}P NMR (DMSO-d$_6$), δ -6.75 ppm; E.S.I. mass (%), 510 [M $^{+\cdot}$] (100%), 512(M+2)(33%); Ana.cal.for $C_{25}H_{21}ClN_3O_3PS$; C, 58.88; H, 4.15; N, 8.24; Found: C, 58.74; H, 4.22; N, 8.32.

Diphenyl 4-(3-(4-chlorophenyl)ureido)phenylphosphoramidate(7e).
Pale yellow solid, yield 73%, M.wt:494, m.p(246-250)^{0}C, IR (KBr), (ν_{max} ,cm^{-1}), 3496(N-H$_{str}$), 1694(C=O), 1595(N-H$_{bend}$), 1397(C-N), 1285(P=O), 1161(P-O-C$_{aromatic}$), 948(P-N); ^{1}H NMR (DMSO-d$_6$), δppm, 8.9(s, 2H, Ar-NH), 8.6(s, 1H, Ar-NH), 7.1-7.6(m, 18H, Ar-H); ^{13}C NMR (DMSO-d$_6$), δ (ppm) 152.4(C-7^1), 150.1(C-1), 138.8(C-1^1), 138.5(C-1^{11}), 133.7(C-4^{11}), 129.9(C-3&C-5), 128.6(C-4^1), 128.5(C-3^{11}&C-5^{11}), 125(C-3^1&C-5^1), 121(C-4), 120.1(C-2^{11}&C-6^{11}), 119.8(C-2&C-6), 118.3(C-2^1&C-6^1); ^{31}P NMR (DMSO-d$_6$), -7.40 ppm; E.S.I.mass (%), 494 [M$^{+\cdot}$](100), 496(M+2)(33%), 281(40%); Ana.cal.for $C_{25}H_{21}ClN_3O_4P$; C, 60.80; H, 4.29; N, 8.51; Found: C, 60.71; H, 4.22; N, 8.45.

Antimicrobial Assay
Antibacterial Assay
Antimicrobial activity of the newly synthesized urea/thiourea derivatives of diphenylphosphoramidate (**4a-e** and **7a-e**) was assayed against two Gram positive bacteria such as *B. substilis* and *Staphylococcus aureus* and two Gram negative bacteria such as *P.aeruginosa* and *E.coli* by agar well diffusion method [31-32]. 200 µg of the tested compounds were dissolved in 1 mL of DMSO solvent. Centrifuged pellets of bacteria from 24 h old culture containing approximately 10^4-10^6 colony forming unit (CFU) per mL was spread on the surface of Muller Hinton Agar (MHA) plates. Nutrient agar medium were prepared by suspended nutrient agar 28 g in 1 liter of distilled water, autoclaved and cooled to 45 °C, then it was seeded with 15 mL of prepared inocula to have 10^6 CFU/mL. Petri dishes were prepared by pouring 10 mL of seeded nutrient agar. Wells were created in medium with the help of a sterile metallic borer and test solution was added. Experimental plates were incubated for 24 h at 37°C and antibacterial activity was defined as the diameter (mm) of the clear inhibition zone formed around the well. Ciprofloxacin was used as standard drug for antibacterial assay.

Determination of Minimum Inhibitory Concentration (MIC)
The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of visible growth after overnight incubation [33]. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to antimicrobial agents and also to monitor the activity of new antimicrobial agents. MIC measurements were performed using a modified agar well diffusion mehod.

Antifungal assay
Antifungal activity of newly synthesized urea/thiourea derivatives of diphenylphosphora midate (**4a-e** and **7a-e**) was tested with *Trichoderma viridae and Aspergillus niger* by the poison plate technique [34]. Tested compounds were dissolved in DMSO before mixing with potato dextrose agar (PDA). The final concentration of the compounds in the medium was fixed at 200 µg/ mL. Two kinds of fungi were incubated in PDA at 25 ±1 °C for 5 days to get new mycelium for antifungal assay, and then a mycelia disk of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. The inoculated plates were incubated at 25 ±1 °C for 5 days. DMSO solvent was added as negative control to determine possible inhibitory activity of the solvent, while miconazole was used as positive control. For each treatment, three replicates were carried out and the mean of the diameter of the inhibition zones was calculated.

RESULTS AND DISCUSSION

Chemistry
The title compounds were synthesized in two steps. In the first step 4,4'diaminodiphenyl sulfone (Dapsone) (**1**)/ 4-amimoaniline (**5**), was reacted with diphenylchlorophosphate (**2**) in presence of triethylamine in dry THF at **60-65 ^{0}C** for **3-5** h to obtain intermediates (**3**) and (**6**). Intermediates(**3**) and (**6**) further treated with various aromatic substituted isocyanates/ thioisocyanates in presence of triethyl amine in dry THF at **60-65 ^{0}C** for **2-4** h to get the urea and thiourea derivatives (**4 a-e & 7a-e**). The progress of the reaction was monitored by TLC using ethyl acetate and hexane (3:2).

The chemical structures of title compounds were characterized by IR, NMR (^{1}H, ^{13}C, ^{31}P), mass spectra and elemental analysis. The title compounds exhibited IR stretching absorptions in the regions 3500-3300, 1740-1680, 1600-1560, 1500-1450, 1430-1400, 1265-1150, 1242-1152 and 970-920 for N-H_{str}, C=O, C=S, N-H_{bend}, C-N, P=O, P-O-$C_{aromatic}$ and P-N respectively. In ^{1}H NMR spectra, aromatic protons of the title compounds (**4a-e** &**7a-e**) gave complex multiplets in the region δ 8.0-6.3, Aromatic N-H protons resonated as singlets in the range of 8-10 ppm. The ^{13} C NMR spectral data of **4a-e & 7a-e** showed characteristic chemical shifts for aromatic carbons. The carbon chemical shifts of C=S, C=O appeared as singlets at 179-185 ppm and152-155 ppm respectively. The conventional ^{13}C numbering for compounds **4a-e** and **7a-e** is represented in Figure 1.The ^{31}P NMR signals appeared as singlets in the region -12 to 12 ppm in all the title compounds.

BIOLOGY

ANTIMICROBIAL ACTIVITY

Biological results reveal that all the synthesized compounds exhibited moderate to high antibacterial activity against both Gram-Positive (*S.aureus*, *B.subtilis*) and Gram negative (*P.aeruginosa*, *E.coli*) (Table 1). Among the synthesized compounds **7b**, **7c**, **4e** and **4d** exhibited good activity against both gram positive and gram negative bacteria as compared with standard ciprofloxacin drug. The compounds **7d**, **7e**, **4b** and **4c** exhibited high activity. On the basis of zone of inhibition against test bacteria four compounds **7b**, **7c**, **4e** and **4d** showed zone of inhibition nearer to the standard drug ciprofloxacin.

Antifungal activity results of title compounds reveal that compounds **7a**, **7b** and **7c** exhibited good antifungal activity as compared with standard miconazole drug. Compound **7e** exhibited moderate activity (Table 2).

For the whole series, MIC of the synthesized compounds ranged between 9-25 µg/mL against both gram positive and gram negative bacteria (Table 3). Among all the synthesized compounds, **7b** exhibited the lowest MIC value at 9-13 µg/mL towards both Gram positive and Gram negative bacteria in comparison to standard drug having MIC 5 µg/mL.

Table.1. Antibacterial activity of title compounds

Compound[a]	Zone of inhibition(mm)			
	B. subtillis	*S. aureus*	*P. aeruginosa*	*E.coli*
7a	11	10	13	10
7b	24	23	25	21
7c	23	23	24	20
7d	18	17	16	14
7e	18	17	17	15
4a	10	09	12	6
4b	18	16	15	13
4c	18.3	16.5	15.5	14.3
4d	22	22	21	19
4e	23	22	22	20
Ciprofloxacin[b]	25	25	26	24

[a] *200 µg/mL*
[b] *200 µg/mL*

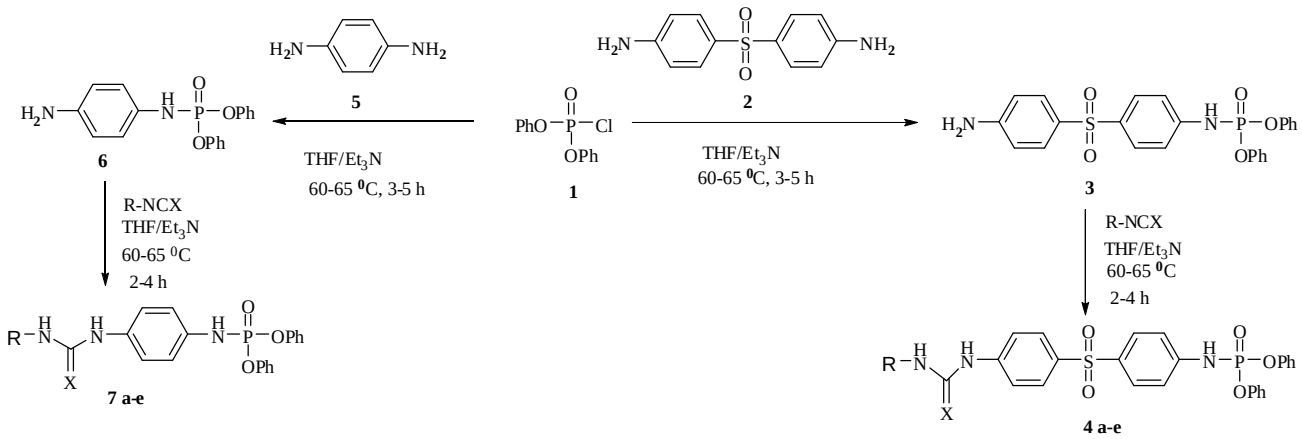
Table.2. Antifungal activity of title compounds

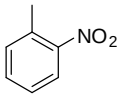
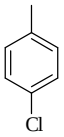
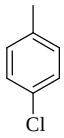
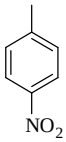
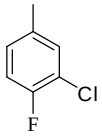
Compound[a]	**Zone of Inhibition (mm)**	
	Trichoderma viridae	***Aspergilllus niger***
7a	17	19
7b	19	17
7c	16	14
7d	11	9
7e	12	12
4a	9	7
4b	9	6
4c	8	7
4d	6	5
4e	10	8
Miconazole[b]	20	20

[a] *200 µg/mL*
[b] *200 µg/mL*

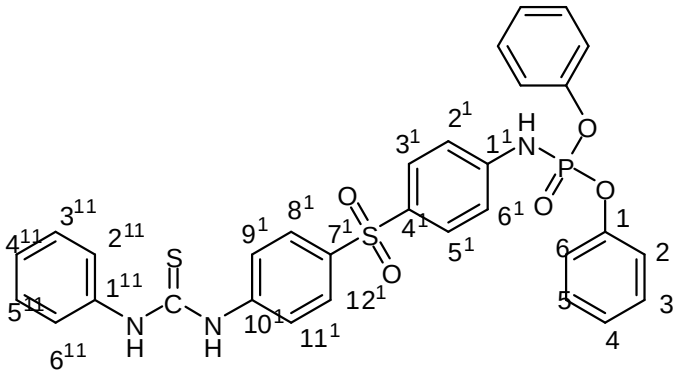
Table.3 Minimum Inhibitory Concentration of the title compounds

Compound	Minimum Inhibition Concentration (MIC) in µg/mL			
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E.coli*
7a	25	24	26	30
7b	9	10	11	13
7c	10	12	13	14
7d	17	19	20	22
7e	18	19	21	24
4a	23	24	25	27
4b	18	20	22	24
4c	16	18	20	22
4d	11	14	14	17
4e	11	13	14	16
Ciprofloxacin	5	5	5	6

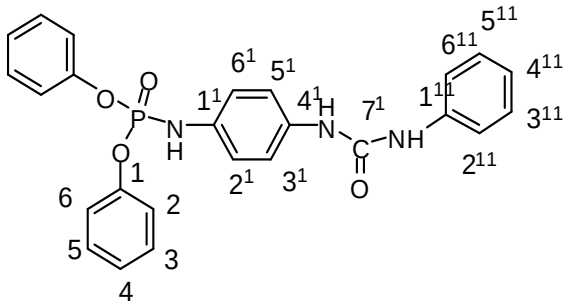


Compound	R	X	Compound	R	X
4a,7a		S	**4d,7b**		O
4b,7d		S	**4c,7e**		O
4e		S	**7c**		O

Scheme-1 Synthesis of title compounds 4a-e & 7a-e



4a-e



7a-e

Figure 1. ^{13}C Numbering of title compounds

CONCLUSION

In conclusion, synthesis of a series of novel urea and thiourea derivatives of diphenylphosphoramidate from 4, 4'-sulfonyldianiline/4-aminoaniline, diphenylchlorophosphate and various aromatic substituted isocyanates /thioisocyanate in presence of triethylamine at 60-65 ^{0}C was accomplished.

All the synthesized compounds exhibited promising antibacterial and antifungal activities. Among all the synthesized compounds (**7b, 7c, 4e** and **4d**) exhibited good antibacterial activity. NO_2 group substituted (**7b, 4d & 4e**) urea and thiourea derivatives exhibited good activity and two halogen containing urea and thiourea derivatives (**7c**) exhibited high activity.

Antifungal activity of synthesized compounds reveals that compounds (**7a, 7b, 7c**) exhibited good antifungal activity and compounds (**7d, 7e, 4e**) exhibited moderate activity.

A definite SAR could not be established due to lack of structural diversity of the investigated compounds and further research is needed to identify lead structure with better activity.

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REFERENCES

[1] M. Grare, M. Mourer, S. Fontanay, J. B. Regnouf-de-Vains, C. Finance, R. E. Duval, *J. Antimicrob. Chemother.*, **2007**, *60*, 575-581.
[2] M.A. Ghannoum, L. B. Rice, *Clinical Microbiology Reviews.*, **1999**, *12*, 501-517.
[3] C. Limban, M.C.B. Chifiriuc, A.V. Missir, I.C. Chiruta, C. Bleotu, *Molecules.*, **2008**, 13, 567-580.
[4] T.K. Venkatachalam, C. Mao, F.M. Uckun, *Bioorg. Med. Chem.*, **2004**, 12, 4275-4280.
[5] B.K. Kaymakcioglu, S. Rollas, E. Ko¨rcegez, F. Aricioglu, *Eur. J. Pharm. Sci.*, **2005**, 26, 97-103.
[6] S.K. Aaramadaka, M.K. Guha, G. Prabhu, S.G. Kini, M. Vijayan, *Chem. Pharm.Bull.*, **2007**, 55, 236-240.
[7] A. Esteves-Souza, K. Pissinate, G. Mda Nascimento, N.F. Grynberg, A. Echevaria, *Bio.org.Med. Chem.*, **2006**, 14, 492-499.
[8] G.M. Coppola, R.E. Damon, J.B. Eskesen, D.S. France, R.Paterniti Jr., *Bioorg.Med. Chem. Lett.*, **2005**, 15, 809-812.
[9] HQ. Li, PC. Lv, T. Yan, HL. Zhu, *Anticancer Agents Med Chem.*, **2009**, 9(4), 471-80.
[10] Cristian Obiol, Gema Alcarrz-Viza, Marta Cascante, Jaime Rubio-Martinez, *Plos one.*, **2012**, 7, 3.
[11] J.M Hamby, P.J. Grohar, A.M. Dohetry, *J. Med. Chem.*, **2001**, 44, 1915
[12] GB. Af Gennas, L. Mologni, S. Ahmed, M. Rajaratnam, O. Marin, N. Lindholm, M. Viltadi, C. Gambacorti-Passerini,L. Scapozza, J. Yli-Kauhaluoma, *Chem.Med.Chem.*, **2011**, 6(9), 1680-92.
[13] J. Nozulak, H.O. Kalkman, P. Floerscheim, D. Hoyer, P. Schoeffer, H.R. Buerki, *J .Med. Chem.*, **1995**, 38, 28-33.
[14] J.E. Audia, D.A. Evrard, G.R. Murdoch, J.J. Droste, J.S. Nissen, K.W. Schenck,P. Fludzinski, V.L. Lucaites, D.L. Nelson, M.L. Cohen, *J. Med. Chem.*, **1996**, 39, 2773-2780.
[15] J. Ding, K. Das, H. Moereels, L. Kaymans, K. Andries, P.A.J. Janssen, S.H. Hughes, E. Arnold, *Nat. Struct. Biol.*, **1995**, 2, 407-415.

[16] E. De Clerq, *II Farmaco*., **1999**, 54, 26-45.
[17] D. S. Sriram, P. Yogeeswari, K. Madhu, *Bioorg. Med. Chem. Lett.*, **2006**, 16, 876.
[18] S-J. Xue, L-P. Duan, S-Y. Ke, J-M. Zhu, *Chin J Org Chem.*, **2004**, 24, 686.
[19] V. K. Madan, A. D. Taneja, V. P. Kudesia, *J Indian Chem Soc.*, **1991**,68, 162.
[20] S. Y. Ke, T. B. Wei, S. J. Xue, L. P. Duan, J. Z. Li, *Indian J Chem.*, **2005**, 44B, 1957.
[21] C. Limbau, M. C. B. Chifiriuc, A. V. Missir, I.C. Chiruta, C. Bleotu, *Molecules.*, **2008**, 13, 567-580.
[22] T. K. Venkatachalam, C. Mao, F. M. Uckun., *Bioorg. Med. Chem.*, **2004**, 12, 4275-4284.
[23] S. K. R. Aaramadaka, M. K. Guha, G. Prabhu, S. G. Kini, M. Vijayan, *Chem. Pharm. Bull.,* **2007**, 55, 236-240.
[24] J. D. Bloom, R. G. Dushin, K. J. Curran, F.Donahue, E. B. Norton, E. Terefenko, T. R. Jonas, A. A.Ross, B. Feld, S. A. Lang, M. J. DiGrandi., *Bioorg. Med. Chem. Lett.*, **2004**, 14, 3401-3406.
[25] P. P. Seth, R. Ranken, D. E. Robinson, S. A. Osgood, L. M. Risen, E. L. Rodgers, M. T. Migawa, E.A. Jefferson, E. E. Swayze, *Bioorg. Med. Chem. Lett.*, **2004**, 14, 5569-5572.
[26] B. K. Kaymakcioglu, S. Rollas, E. Körcegez, F. Aricioglu, *Eur. J. Pharm. Sci*., **2005**, 26, 97-103.
[27] C. Flader, J. Liu, R.F. Borch, *J. Med. Chem.*, **2000**, 43, 3157.
[28] G. Zon, *Pharm Res.*, **1988**, *5, 549.*
[29] D. Petersen, M. Marcolini, L. Bernadi, F. Fini, P. R. Herrera, V. Sgarzani, A. Ricci, *J. Org. Chem*., **2006**, 71, 6269.
[30] R. Carran, A. Maran, J. M. Montero, L.Fernadozlago, A. Dominguez, *Plants Med. Phyto*.. **1987**, 21,195-204.
[31] S.U.Kazmi, S.N.Ali, S. A.Jamal, Atta-ur-Rehman, *J.Pharm.Sci.*, **1991**, 4, 113-118.
[32] Atta-ur-Rehman, M.I.Choudhary, W.J.Thomson, Bioassay Techniques for Drug Development, *Harwood Academic Publishers.,* Amsterdam, **2001**.
[33] D. Greenwood, R. Slack, J. Peutherer, Medical Microbiology, in: A Guide to Microbial Infections:Pathogenesis, Immunity, Laboratory Diagnosis and Control, ELST Publishers, Edinburgh, **1997**, 15th edition.
[34] Syed Raseed, Katla Venkataramana, Kuruva Chandrasekhar, Gemmalamadugu Fareeda and Chamarti Naga Raju. *Arch.Pharm.Chem.Life Sci*., **2012**, 345, 294-301.