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$SiO_2/ZnCl_2$ catalyzed α-aminophosphonates and phosphonated N-(substituted phenyl) sulfonamides of 2-aminothiophene: Synthesis and biological evaluation

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ABSTRACT

In the present report, an expeditious green synthetic approach was developed for the synthesis of new α-aminophosphonates ***6(a-f)*** *of multisubstituted 2-aminothiophene in good yields through one-pot three component reaction (Kabachnik-Fields reaction) catalyzed by silica supported $ZnCl_2$ under solvent-free conditions. In vitro antimicrobial and antioxidant activities were evaluated and the data revealed that the compound* ***6e*** *exhibited significantactivities. Again, this active α-aminophosphonate* ***6e*** *was proceeded as precursor and a series of novel N-(substituted phenyl) sulfonamidoα-amino phosphonate derivatives, ethyl 5-[N-(diethoxyphosphoryl)(2-hydroxyphenyl)methyl)substitutedphenylsulfonamido]-4-cyano-3-methylthiophene-2-carboxylates* ***8(a-e)*** *were accomplished by reactingwith active functionalized phenylsulfonyl chlorides. In vitro antimicrobial and antioxidant activities of these synthesized compounds* ***8(a-e)*** *were screened at different concentration. IC_{50} values were also determined. It was found that there is enhancement in antimicrobial activity and no significant enhancement in antioxidant activity in compounds* ***8(a-e)*** *when compared with active α-aminophosphonate* ***6e****.*

Keywords: Silica supported $ZnCl_2$, Neat reaction conditions, α-Aminophosphonates, *N*-substituted phenyl sulfonamido α-amino phosphonates, Antimicrobial activity, Antioxidant activity.

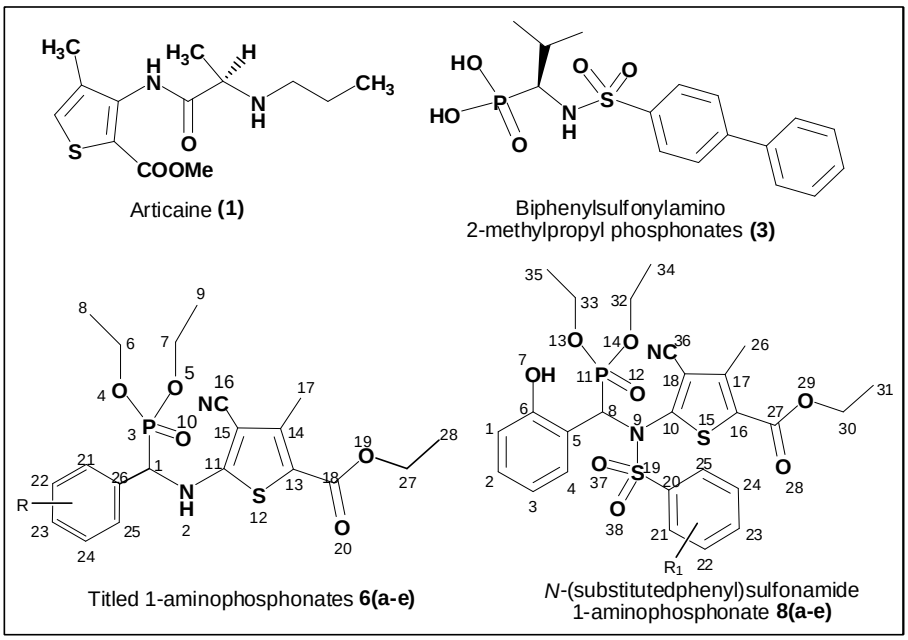
INTRODUCTION

Extensive evidence has been accumulated in the literature to demonstrate the efficacy of the five membered heterocyclic molecules for their versatile biological properties in various fields. In fact, sulfur doped heterocycles have gained additional prominence after discovery of sulfur drugs and mesoionic compounds. Among, multisubstituted 2-aminothiophene and thiophenes are privileged motifs in numerous drug molecules for example articaine **(1)**(**Fig.1**) is the most widely used local dental anaesthetic in several European countries and this kind of compounds have potent antioxidant and anti-inflammatory [1-2], antibacterial [3], antifungal [4], anti-HIV [5], anti-breast cancer [6] and antitubercular [7] activities. In view of that multisubstituted 2-aminothiophenes can be utilized as building blocks in the synthesis of new class of α-aminophosphonateanalogues with a hope that there will exhibit potent biological activity.

Phosphorus compounds are biologically active molecules and exhibit a wide range of applications in the area of industrial, agricultural and medicinal chemistry. α-Aminophosphonate esters/aminophosphonic acids as mimics of the α-amino acids are showing a great deal of interest in medicinal chemistry due to their broad spectrum of

biological properties as potent antibiotics [8], antiviral agents [9], herbicidal activities [10], antitumor agents [11] and enzyme inhibitors [12]. Kabachnik–Fields reaction is the most convenient and widely used method to synthesize α-aminoalkylphosphonates, catalyzed by a base or Lewis acids. The traditional Lewis acid catalyzed Kabachnik-Fields reaction has provided useful method for the preparation of α-aminophosphonate esters. Some of them are indium(III) chloride [13], $TaCl_5$-SiO_2 [14], $BiCl_3$ [15], $FeCl_3$ [16] and $YbCl_3$ [17]. Nevertheless, these catalysts have some drawbacks like long reaction time, low yield, undesired products are obtained, expensive catalyst, generate large amount of waste and rigorous reaction conditions. Recently, several organic transformations are effectively catalyzed by silica gel supported Lewis acid catalysts in organic synthesis [18]. The reagent dispersed on the surface of a support is improved the effective surface area of the reagent significantly and hence they are expected to perform more effectively than the individual reagents.Hence, inexpensive, reusable catalysts are needed to enable the one-pot synthesis of α-aminophosphonates.Herein, a green synthetic approach was developed for the synthesis of new α-aminophosphonates of2-aminothiophene in high yields through one-pot three component reaction (Kabachnik-Fields reaction) catalyzed by silica supported $ZnCl_2$ under solvent-free conditions and the catalyst was reused for five times.

Fig-1 Some biologically active thiophene and phosphorus containing molecules



Further, the extensive interest is mounting to the synthesis of substituted phosphoryl derivatives due to their biological activities with broad application as enzyme inhibitors, antimetabolites and antibiotics [19].Recently, few credentials demonstrated that 1-ureidophosphonates have shown promising antiviral activity against TMV [20], as insecticides [21] and α-biphenylsulfonylamino 2-methylpropyl phosphonate**(2)**(**Fig.1**) analogues attain more potency against several MPIs [22]. Considering thebiological interest of the substituted phosphoryl derivatives, we turned our attention to prepare novel *N*-(substitutedphenyl)sulfonamido α-aminophosphonate derivatives by incorporating the functionalized phenylsulfonyl chlorides at –NH position of the newly synthesized biologically active α-aminophosphonate**6e**.

MATERIALS AND METHODS

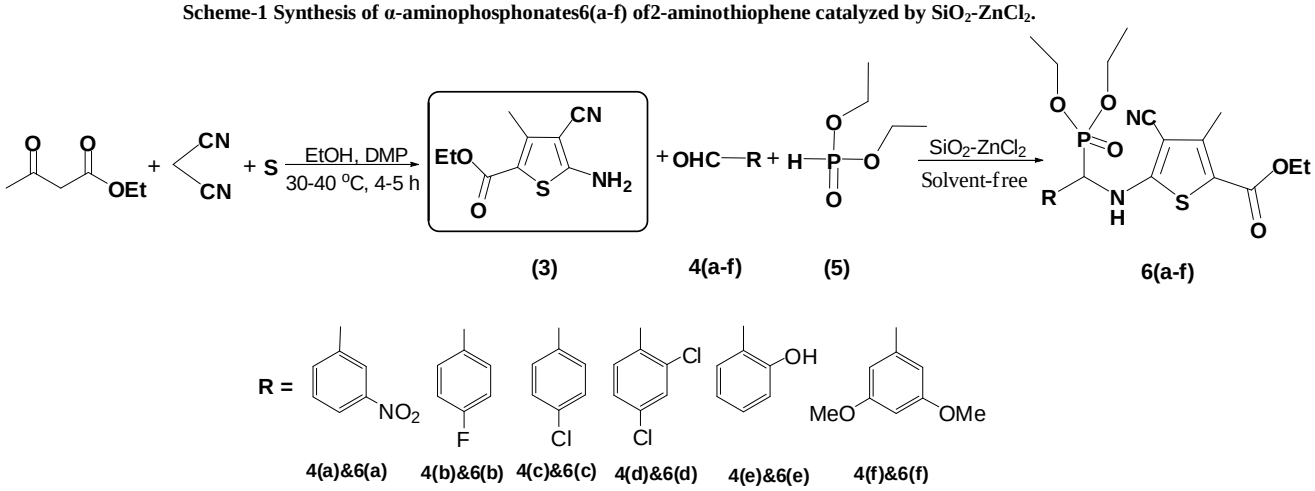
All chemicals used were purchased from Aldrich. Solvents were distilled from the appropriate drying agents and stored under nitrogen atmosphere. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points were determined in open capillaries on Gunamelting point apparatus and are uncorrected. ^{1}H NMR, ^{31}P NMR and ^{13}C NMR spectra were recorded on Bruker AV-500 spectrometer. TMS was used as the internal standard for ^{1}H and ^{13}C NMR spectra and H_3PO_4 as the external standard for ^{31}P NMR spectra and chemical shifts were recorded in parts per million (ppm). Multiplicities are shown as the abbreviations: s

(singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet). . E.S.I mass spectra were recorded on a MLP 2103 mass spectrometer. Elemental analysis was performed on Thermo Finnigan Flash 1112 instrument. The silica gel (200 mesh) was used in column chromatography for purification of the synthesized compounds. The numbering was given to the title compound for assigning the proper spectral characterization (**Fig-1**).

General procedure for the synthesis of α-aminophosphonates 6(a-f) (See Scheme-1)

Ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate **(3)** (210 mg, 1 mmol), *m*-nitro benzaldehyde**(4a)** (151 mg, 1 mmol), diethylphosphite**(5)** (0.16 mL, 1.2 mmol) and SiO_2-$ZnCl_2$ (15 mol%) were taken into flat-bottomed flask and stirred vigorously at 40 ^oC for 3 h. After completion of the reaction as monitored by TLC, the reaction mixture was dissolved in DCM (10 mL) and filtered-off to remove the catalyst, SiO_2-$ZnCl_2$. The filtrate was concentrated under vacuum and the crude product was employed for column chromatography to afford pure, ethyl4-cyano-5-[(diethoxyphosphoryl)(3-nitrophenyl)methylamino]-3-methylthiophene-2-carboxylate **(6a)**. 30% ethyl acetate and petroleum ether was used as eluent to run the column. The residue i.e catalyst was washed with $CHCl_3$ to remove stains and dried in oven and reused further reaction. The same successful procedure was employed for the preparation all the compounds **6(a-f)** (**Table-9**).

Scheme-1 Synthesis of α-aminophosphonates6(a-f) of2-aminothiophene catalyzed by SiO_2-$ZnCl_2$.



General procedure for the synthesis of *N*-substituted phenyl sulfonamido α-amino phosphonates 8(a-e) (See Scheme-2)

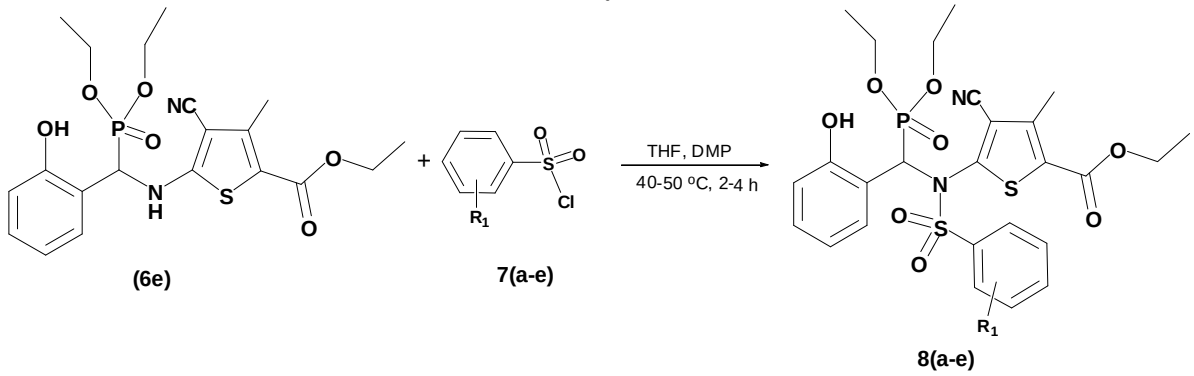
The newly synthesized aminophosphonate, ethyl 4-cyano-5-[(diethoxyphosphoryl)(2-hydroxyphenyl)methylamino]-3-methyl thiophene-2-carboxylate **(6e)** (317 mg, 0.7 mmol), 4-nitrobenzene-1-sulfonyl chloride **(7c)** (155 mg, 0.7 mmol) and dimethyl piperazine (DMP) (0.7 mmol) as a base were taken into a round-bottomed flask containing 10 mL of THF. The reaction mixture was refluxed for 3 h and the progress of the reaction was checked on TLC. After completion of the reaction, the salt was filtered-off and the filtrate was concentrated under vacuum to get crude product. It was purified by column chromatography using 35% ethyl acetate and petroleum ether as eluent to afford pure ethyl 4-cyano-5-[*N*-((diethoxyphosphoryl)(2-hydroxyphenyl)methyl)-4-nitrophenyl sulfonamido]-3-methylthiophene-2-carboxylate **(8c)**. The same procedure was used for the preparation of the remaining compounds **8(a-e)**.

Ethyl 4-cyano-5-[(diethoxyphosphoryl)(3-nitrophenyl)methylamino]-3-methylthiophene-2-carboxylate (6a).

Dark yellow solid; Yield: 93.4;m.p. 145-146 ^oC. IR (KBr, υ cm^{-1}): 3354 (-N-H, str), 3067 (=C-H, str), 2986 (-C-H, str), 2234 (-CN, str), 1691 (-C=O, str), 1450 (-N=O, str), 1277 (-P=O, str); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.32 (t, J = 6.8 Hz, 3H, -P-O-CH_2-$\underline{CH_3}$), 1.39 (t, J = 7.2 Hz, 3H, -P-O-CH_2-$\underline{CH_3}$), 1.54 (t, J = 7.6 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.64 (s, 3H, thiophene-$\underline{CH_3}$), 3.73-3.95 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.13-4.18 (q, J = 7.2 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 4.57-4.65 (dd, J_{CH-P} = 17.4, 6.4 Hz, 1H, -P-$\underline{CH}$-NH-), 5.82 (s, 1H, -CH-N$\underline{H}$-), 7.56-7.79 (m, 2H, Ar-H), 8.14 (d, J = 5.6 Hz, 1H, Ar-H), 8.47 (s, 1H, Ar-H) ppm; ^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 10.9 (C_{17}), 17.4 (C_{28}), 18.8 ($C_{9,8}$), 50.3 (d, C_1), 59.8 (C_{27}), 63.2 (d, J_{P-O-C} = 31.9 Hz, $C_{6,7}$), 89.5 (C_{15}), 117.0 (C_{16}), 124.8 (C_{23}), 125.4 (C_{25}), 129.3 (C_{22}), 135.8 (C_{21}), 137.0 (C_{26}), 140.2 (C_{14}), 144.8 (C_{24}), 152.1 (C_{13}), 161.6 (C_{18}), 174.4 (C_{11}) ppm; ^{31}P-NMR ($CDCl_3$, 125

MHz): δ 20.00. MS (Positive mode), m/z (%): 482 ($M+H^+$, 100%), 437 ($M+H^+$-45, 48%). Anal.Calcd.for $C_{20}H_{24}N_3O_7PS$: C, 49.89; H, 5.02; N, 8.73 found: C, 49.52; H, 4.96; N, 8.71%.

Scheme-2 Synthesis of phosphonated*N*-(substitutedphenyl)sulfonamido derivatives of ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate.



(6e) 7(a-e) 8(a-e)

Product	7a&8a	7b&8b	7c&8c	7d&8d	7e&8e
R_1-	4-F	4-Br	4-NO_2	3-Cl, 4-NO_2	2-OH, 5-NO_2

Ethyl 4-cyano-5-[(diethoxyphosphoryl)(4-fluorophenyl)methylamino]-3-methylthiophene-2-carboxylate (6b).
Light brown solid; Yield: 90.8;m.p. 157-159 °C. IR (KBr, υ cm^{-1}): 3352 (-N-H, str), 3068 (=C-H, str), 2980 (-C-H, str), 2243 (-CN, str), 1694 (-C=O, str), 1263 (-P=O, str), 1046 (-C-F, str); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.28 (t, J = 6.4 Hz, 3H, -P-O-CH_2-$\underline{CH_3}$), 1.31 (t, J = 6.4 Hz, 3H, -P-O-CH_2-$\underline{CH_3}$), 1.52 (t, J = 7.2 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.53 (s, 3H, thiophene-$\underline{CH_3}$), 3.64-3.71 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.08-4.14 (q, J = 7.2 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 4.51-4.58 (dd, J_{CH-P} = 16.8, 6.0 Hz, 1H, -P-$\underline{CH}$-NH-), 5.36 (s, 1H, -CH-N$\underline{H}$-), 7.28 (d, J = 5.6 Hz, 2H, Ar-H), 7.34 (d, J = 5.6 Hz, 2H, Ar-H) ppm; ^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 11.6 (C_{17}), 17.1 (C_{28}), 17.9 ($C_{9,8}$), 50.1 (d, C_1), 60.4 (C_{27}), 63.4 (d, J_{P-O-C} = 29.2 Hz, $C_{6,7}$), 91.2 (C_{15}), 117.3 ($C_{22,24}$), 118.7 (C_{16}), 131.4 ($C_{21,25}$), 135.6 (C_{26}), 142.8 (C_{14}), 152.2 (C_{13}), 158.54 (C_{23}), 162.7 (C_{18}), 174.8 (C_{11}) ppm; ^{31}P-NMR ($CDCl_3$, 125 MHz): δ 22.32. MS (Positive mode), m/z (%): 455 ($M+H^+$, 100%), 410 ($M+H^+$-45, 59%).

Ethyl 5-[(4-chlorophenyl)(diethoxyphosphoryl)methylamino]-4-cyano-3-methylthiophene-2-carboxylate (6c).
Brown solid; Yield: 89.0; m.p. 136-138 °C. IR (KBr, υ cm^{-1}): 3352 (-N-H, str), 3065 (=C-H, str), 2982 (-C-H, str), 2234 (-CN, str), 1694 (-C=O, str), 1268 (-P=O, str), 842 (-C-Cl, str); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.26 (t, J = 6.8 Hz, 3H, -P-O-CH_2-$\underline{CH_3}$), 1.32 (t, J = 6.4 Hz, 3H, -P-O-CH_2-$\underline{CH_3}$), 1.48 (t, J = 6.8 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.58 (s, 3H, thiophene-$\underline{CH_3}$), 3.78-3.83 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.13-4.17 (q, J = 6.8 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 4.43-4.50 (dd, J_{CH-P} = 15.4, 6.4 Hz, 1H, -P-$\underline{CH}$-NH-), 5.28 (s, 1H, -CH-N$\underline{H}$-), 7.19 (d, J = 5.2 Hz, 2H, Ar-H), 7.26 (d, J = 5.6 Hz, 2H, Ar-H) ppm; ^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 10.2 (C_{17}), 17.4 (C_{28}), 18.2 ($C_{9,8}$), 50.3 (d, C_1), 60.4 (C_{27}), 62.7 (d, J_{P-O-C} = 28.8 Hz, $C_{6,7}$), 88.5 (C_{15}), 117.2 (C_{16}), 129.6 ($C_{21,25}$), 134.2 ($C_{22,24}$), 135.2 (C_{23}), 136.1 (C_{26}), 141.9 (C_{14}), 154.7 (C_{13}), 163.1 (C_{18}), 175.3 (C_{11}) ppm; ^{31}P-NMR ($CDCl_3$, 125 MHz): δ 21.62. MS (Positive mode), m/z (%): 473 ($M+H^+$+2, 32%), 471 ($M+H^+$, 100%).

Ethyl 4-cyano-5-[(2,4-dichlorophenyl) (diethoxyphosphoryl) methylamino]-3-methyl thiophene-2-carboxylate (6d).
Brown solid; Yield 88.5; m.p. 131-133 °C. IR (KBr, υ cm^{-1}): 3358 (-N-H, str), 3058 (=C-H, str), 2980 (-C-H, str), 2236 (-CN, str), 1692 (-C=O, str), 1264 (-P=O, str), 838 (-C-Cl, str); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.22 (t, J = 6.4 Hz, 3H, -P-O-CH_2-$\underline{CH_3}$), 1.28 (t, J = 6.4 Hz, 3H, -P-O-CH_2-$\underline{CH_3}$), 1.46 (t, J = 6.8 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.61 (s, 3H, thiophene-$\underline{CH_3}$), 3.72-3.78 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.20-4.26 (q, J = 6.4 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 4.36-4.43 (dd, J_{CH-P} = 12.8, 6.0 Hz, 1H, -P-$\underline{CH}$-NH-), 5.03 (s, 1H, -CH-N$\underline{H}$-), 7.16 (d, J = 5.6 Hz, 1H, Ar-H), 7.19 (d, J = 5.6 Hz, 1H, Ar-H), 7.84 (s, 1H, Ar-H) ppm; ^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 10.7 (C_{17}), 17.8 (C_{28}), 18.3 ($C_{9,8}$), 51.1 (d, C_1), 61.1 (C_{27}), 63.4 (d, J_{P-O-C} = 30.2 Hz, $C_{6,7}$), 90.2 (C_{15}), 118.1 (C_{16}), 128.1 (C_{22}), 131.5 (C_{21}), 133.4 (C_{24}), 135.4 (C_{25}), 137.1 (C_{23}), 140.8 (C_{26}), 141.7 (C_{14}), 153.8 (C_{13}), 162.7 (C_{18}), 175.1 (C_{11}) ppm; ^{31}P-NMR ($CDCl_3$, 125 MHz): δ 22.18. MS (Positive mode), m/z (%): 507 ($M+H^+$+2, 62%), 505 ($M+H^+$, 100%).

Ethyl 4-cyano-5-[(diethoxyphosphoryl) (2-hydroxyphenyl) methylamino]-3-methyl thiophene-2-carboxylate (6e).
Light green solid; Yield: 89.6;m.p. 182-183^{o}C. IR (KBr, υ cm^{-1}): 3486 (-O-H str), 3356 (-N-H, str), 3064 (=C-H, str), 2980 (-C-H, str), 2250 (-CN, str), 1690 (-C=O, str), 1258 (-P=O, str); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.34 (t, J = 6.8 Hz, 6H, -P-(O-CH_2-$\underline{CH_3}$)$_2$), 1.40 (t, J = 7.2 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.43 (s, 3H, thiophene-$\underline{CH_3}$), 4.17-4.22 (q, J = 6.8 Hz, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.27-4.32 (q, J = 7.2 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 4.35 (d, J_{CH-P} = 8.4 Hz, 1H, -P-$\underline{CH}$-NH-), 5.36 (s, 1H, -CH-N$\underline{H}$-), 6.90-6.98 (m, 2H, Ar-H), 7.00-7.43 (m, 2H, Ar-H), 9.86 (s, 1H, Ar-OH) ppm; ^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 10.2 (C_{17}), 16.7 (C_{28}), 18.2 ($C_{9,8}$), 53.6 (C_1), 62.4 (C_{27}), 63.1 (d, J_{P-O-C} = 30.2 Hz, $C_{6,7}$), 88.9 (C_{15}), 118.7 (C_{16}), 119.2 (C_{22}), 123.8 (C_{26}), 125.3 (C_{24}), 131.6 (C_{25}), 132.2 (C_{23}), 145.7 (C_{14}), 152.9(C_{13}), 157.4 (C_{21}), 163.1 (C_{18}), 176.8 (C_{11}) ppm; ^{31}P-NMR ($CDCl_3$, 125 MHz): δ 21.28. MS (Positive mode), m/z (%): 453 ($M+H^+$, 100%).

Ethyl 4-cyano-5-((diethoxy phosphoryl) (3,5-dimethoxy phenyl) methylamino)-3-methyl thiophene-2-carboxylate (6f).
Brown solid; Yield: 89.7;m.p. 176-178^{o}C. IR (KBr, υ cm^{-1}): 3368 (-N-H, str), 3042 (=C-H, str), 2982 (-C-H, str), 2246 (-CN, str), 1694 (-C=O, str), 1256 (-P=O, str); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.31 (t, J = 7.2 Hz, 6H, -P-(O-CH_2-$\underline{CH_3}$)$_2$), 1.37 (t, J = 7.6 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.48 (s, 3H, thiophene-$\underline{CH_3}$), 3.17 (s, 6H, (-OCH_3)$_2$), 3.96-4.04 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.09-4.14 (q, J = 7.6 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 4.26 (dd, J_{CH-P} = 10.4, 6.0 Hz, 1H, -P-$\underline{CH}$-NH-), 5.14 (s, 1H, -CH-N$\underline{H}$-), 6.42 (s, 1H, Ar-H), 6.98 (s,1H, Ar-H), 7.12 (s, 1H, Ar-H) ppm;^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 10.8 (C_{17}), 17.1 (C_{28}), 18.8 ($C_{9,8}$), 52.6 (C_1), 57.6 (-O$\underline{C}$)$_2$), 61.4 (C_{27}), 63.1 (d, J_{P-O-C} = 27.8 Hz, $C_{6,7}$), 89.3 (C_{15}), 104.6 (C_{23}), 111.4 (C_{21}), 112.2 (C_{25}), 117.5 (C_{16}), 139.6 (C_{26}), 145.1 (C_{14}), 153.2 (C_{13}), 160.4 (C_{22}), 160.7 (C_{24}), 162.3 (C_{18}), 173.9 (C_{11}) ppm;^{31}P-NMR ($CDCl_3$, 125 MHz): δ 20.64. MS (Positive mode), m/z (%): 497 ($M+H^+$, 100%).

Ethyl 4-cyano-5-(N-((diethoxyphosphoryl) (2-hydroxyphenyl) methyl)-4-fluorophenyl sulfonamido)-3-methyl thiophene-2-carboxylate (8a).
Brown solid; Yield: 82.6; m.p. 152-153 ^{o}C. IR (KBr, υ cm^{-1}): 3465 (-O-H, str), 3086 (=C-H, str), 2984 (-C-H, str), 2278 (-CN, str), 1695 (-C=O, str), 1354 (-SO_2, asymstr), 1231 (-P=O, str), 1135 (-SO_2, symstr), 1018 (-C-F, str); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.14 (t, J = 6.4 Hz, 6H, -P-(O-CH_2-$\underline{CH_3}$)$_2$), 1.17 (t, J = 6.8 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.57 (s, 3H, thiophene-$\underline{CH_3}$), 4.22-4.28 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.30-4.35 (q, J = 6.0 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 5.14 (d, J_{CH-P} = 12.0 Hz, 1H, -CH-P-), 6.81-6.83 (m, 2H, Ar-H), 7.10-7.13 (m, 2H, Ar-H), 7.38-7.42 (m, 2H, Ar-H), 7.51-7.58 (m, 2H, Ar-H), 9.86 (brs, 1H, Ar-OH) ppm; ^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 11.2 (C_{26}), 16.7 (C_{31}), 18.7 ($C_{34,35}$), 49.2 (d, J_{C-P} = 6.8 Hz, C_8), 63.1 (C_{30}), 65.9 (d, J_{P-O-C} = 28.6 Hz, $C_{32,33}$), 93.2 (C_{18}), 116.3 (C_1), 118.1 (C_{36}), 119.1 ($C_{22,24}$), 122.5 (C_3), 123.8 (C_5), 128.1 (C_2), 128.4 (C_4), 131.4 ($C_{21,25}$), 136.9 (C_{20}), 140.8 (C_{17}), 145.3 (C_{10}), 151.1 (C_{16}), 157.1 (C_6), 163.3 (C_{27}), 165.5 (C_{23}) ppm;^{31}P-NMR ($CDCl_3$, 125 MHz): δ 19.59. MS (Positive mode), m/z (%): 611 ($M+H^+$, 64%), 566 ($M+H^+$-45, 23%), 452 ($M+H^+$-159, 100%).

Ethyl 5-(4-bromo-N-((diethoxyphosphoryl) (2-hydroxyphenyl) methyl) phenyl sulfonamido)-4-cyano-3-methyl thiophene-2-carboxylate (8b).
Light green solid; Yield: 84.7; m.p. 174-176 ^{o}C. IR (KBr, υ cm^{-1}): 3477 (-O-H, str), 3065 (=C-H, str), 2984 (-C-H, str), 2298 (-CN, str), 1705 (-C=O, str), 1348 (-SO_2, asymstr), 1232 (-P=O, str), 1122 (-SO_2, symstr), 692 (-C-Br, str); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.16 (t, J = 6.4 Hz, 6H, -P-(O-CH_2-$\underline{CH_3}$)$_2$), 1.17 (t, J = 6.4 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.62 (s, 3H, thiophene-$\underline{CH_3}$), 4.20-4.28 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.32-4.37 (q, J = 5.6 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 5.21 (d, J_{CH-P} = 12.0 Hz, 1H, -CH-P-), 6.73-6.69 (m, 2H, Ar-H), 7.16-7.23 (m, 2H, Ar-H), 8.07-8.17 (m, 3H, Ar-H), 10.01 (brs, 1H, Ar-OH) ppm; ^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 8.8 (C_{26}), 16.1 (C_{31}), 18.2 ($C_{34,35}$), 45.5 (d, J_{C-P} = 36.6 Hz, C_8), 52.2 (C_{30}), 65.7 (d, J_{P-O-C} = 37.7 Hz, $C_{32,33}$), 93.1 (C_{18}), 113.9 (C_1), 116.4 (C_{36}), 125.2 (C_3), 127.7 (C_5), 127.8 (C_{23}), 129.9 (C_2), 130.2 (C_4), 131.3 ($C_{21,25}$), 132.3 ($C_{22,24}$), 137.2 (C_{20}), 148.6 (C_{10}), 150.0 (C_{17}), 156.5 (C_{16}), 158.3 (C_6), 167.7 (C_{27}), ppm; ^{31}P-NMR ($CDCl_3$, 125 MHz): δ 21.62. MS (Positive mode), m/z (%): 673 ($M+H^+$+2, 54%), 671 ($M+H^+$, 51%), 452 ($M+H^+$-219, 100%). Anal.Calcd.for $C_{26}H_{28}BrN_2O_8PS_2$: C, 46.50; H, 4.20; N, 4.17 found: C, 46.48; H, 4.16; N, 4.15%.

Ethyl 4-cyano-5-[N-((diethoxy phosphoryl) (2-hydroxy phenyl) methyl)-4-nitrophenyl sulfonamido]-3-methyl thiophene-2-carboxylate (8c).
Light yellow solid; Yield: 89.3; m.p. 123-125 ^{o}C. IR (KBr, υ cm^{-1}): 3468 (-O-H, str), 3092 (=C-H, str), 2986 (-C-H, str), 2323 (-CN, str), 1693 (-C=O, str), 1462 (-N=O, str), 1351 (-SO_2, asymstr), 1230 (-P=O, str), 1147 (-SO_2, symstr); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.15 (t, J = 6.0 Hz, 6H, -P-(O-CH_2-$\underline{CH_3}$)$_2$), 1.19 (t, J = 6.8 Hz, 3H, O=C-O-

CH_2-$\underline{CH_3}$), 2.49 (s, 3H, thiophene-$\underline{CH_3}$), 4.20-4.29 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.31-4.35 (q, J = 6.0 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 5.18 (d, J_{CH-P} = 12.8 Hz, 1H, -CH-P-), 6.67-6.74 (m, 2H, Ar-H), 7.10-7.12 (m, 2H, Ar-H), 7.13-7.20 (m, 4H, Ar-H), 10.9 (brs, 1H, Ar-OH) ppm; 13C-NMR ($CDCl_3$, 100.61 MHz) : δ 10.9 (C_{26}), 15.58 (C_{31}), 17.3 ($C_{34,35}$), 49.2 (d, J_{C-P} = 5.1 Hz, C_8), 61.9 (C_{30}), 66.4 (d, J_{P-O-C} = 31.2 Hz, $C_{32,33}$), 94.9 (C_{18}), 114.1 (C_1), 117.32 (C_{36}), 123.6 (C_3), 125.4 (C_5), 127.8 ($C_{22,24}$), 128.3 ($C_{21,25}$), 128.4 (C_2), 129.8 (C_4), 143.9 (C_{17}), 146.0 (C_{10}), 150.3 (C_{20}), 153.4 (C_{23}), 155.8 (C_{16}), 157.3 (C_6), 163.6 (C_{27}) ppm; ^{31}P-NMR ($CDCl_3$, 125 MHz): δ 20.74. MS (Positive mode), m/z (%): 638 ($M+H^+$, 58%), 593 ($M+H^+$-45, 18%), 452 ($M+H^+$-186, 100%). Anal.Calcd.for $C_{26}H_{28}N_3O_{10}PS_2$: C, 48.98; H, 4.43; N, 6.59 found: C, 48.86; H, 4.40; N, 6.51%.

.

Ethyl 5-(3-chloro-N-((diethoxyphosphoryl)(2-hydroxyphenyl)methyl)-4-nitrophenyl sulfonamido)-4-cyano-3-methylthiophene-2-carboxylate(8d).

Dark brown solid; Yield: 87.2; m.p. 145-147 ^oC. IR (KBr, υ cm^{-1}): 3487 (-O-H, str), 3084 (=C-H, str), 2994 (-C-H, str), 2268 (-CN, str), 1684 (-C=O, str), 1451 (-N=O, str), 1362 (-SO_2, asymstr), 1232 (-P=O, str), 1148 (-SO_2, symstr), 874 (-C-Cl, str); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.13 (t, J = 7.2 Hz, 6H, -P-(O-CH_2-$\underline{CH_3}$)$_2$), 1.17 (t, J = 6.8 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.66 (s, 3H, thiophene-$\underline{CH_3}$), 4.11-4.29 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.34-4.40 (q, J = 6.8 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 5.63 (s, 1H, -CH-P-), 6.73-6.69 (m, 2H, Ar-H), 8.07 (d, J = 8.8 Hz, 2H, Ar-H), 8.12 (d, J = 8.8 Hz, 2H, Ar-H), 8.41-8.69 (m, 2H, Ar-H), 10.07 (s, 1H, Ar-OH) ppm; ^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 10.8 (C_{26}), 15.6 (C_{31}), 18.2 ($C_{34,35}$), 51.8 (d, J_{C-P} = 7.2 Hz, C_8), 64.3 (C_{30}), 67.5 (d, J_{P-O-C} = 31.9 Hz, $C_{32,33}$), 91.8 (C_{18}), 116.8 (C_1), 117.2 (C_{36}), 121.8 (C_3), 123.8 (C_5), 126.2 (C_{22}), 127.5 (C_{21}), 127.9 (C_{25}), 128.8 (C_2), 129.6 (C_4), 131.7 (C_{24}), 142.6 (C_{17}), 147.1 (C_{10}), 148.4 (C_{20}), 150.4 (C_{23}), 153.6 (C_{16}), 155.8 (C_6), 164.2 (C_{27}), ppm; ^{31}P-NMR ($CDCl_3$, 125 MHz): δ 23.52. MS (Positive mode), m/z (%):674 ($M+H^+$+2, 21%), 672 ($M+H^+$, 59%), 627 ($M+H^+$-45, 24%), 452 ($M+H^+$-220, 100%).

Ethyl 4-cyano-5-(N-((diethoxyphosphoryl)(2-hydroxyphenyl)methyl)-2-hydroxy-5-nitrophenyl sulfonamido)-3-methylthiophene-2-carboxylate (8e).

Yellow solid, Yield 84.82. Mol. Wt: 653.09, mp 167-169 ^oC. IR (KBr, υ cm^{-1}): 3475, 3406 (-O-H, str), 3070 (=C-H, str), 2932 (-C-H, str), 2299 (-CN, str), 1687 (-C=O, str), 1450 (-N=O, str), 1390 (-SO_2, asymstr), 1255 (-P=O, str), 1163 (-SO_2, symstr); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.18 (t, J = 7.2 Hz, 6H, -P-(O-CH_2-$\underline{CH_3}$)$_2$), 1.34 (t, J = 7.6 Hz, 3H, O=C-O-CH_2-$\underline{CH_3}$), 2.43 (s, 3H, thiophene-$\underline{CH_3}$), 4.02-4.07 (m, 4H, -P-(O-$\underline{CH_2}$-CH_3)$_2$), 4.17-4.22 (q, J = 6.8 Hz, 2H, O=C-O-$\underline{CH_2}$-CH_3), 5.36 (s, 1H, -CH-P-), 6.92-7.41 (m, 5H, Ar-H), 8.00 (d, J = 5.2 Hz, 1H, Ar-H), 8.61 (s, 1H, Ar-H), 9.83 (s, 1H, Ar-OH), 11.82 (s, 1H, Ar-OH) ppm; ^{13}C-NMR ($CDCl_3$, 100.61 MHz) : δ 9.9 (C_{26}), 15.6 (C_{31}), 18.2 ($C_{34,35}$), 51.8 (d, J_{C-P} = 7.2 Hz, C_8), 64.3 (C_{30}), 67.5 (d, J_{P-O-C} = 31.9 Hz, $C_{32,33}$), 91.8 (C_{18}), 116.8 (C_1), 117.2 (C_{36}), 121.8 (C_3), 123.8 (C_5), 126.2 (C_{22}), 127.5 (C_{21}), 127.9 (C_{25}), 128.8 (C_2), 129.6 (C_4), 131.7 (C_{24}), 142.6 (C_{17}), 147.1 (C_{10}), 148.4 (C_{20}), 150.4 (C_{23}), 153.6 (C_{16}), 155.8 (C_6), 164.2 (C_{27}), ppm; ^{31}P-NMR ($CDCl_3$, 125 MHz): δ 23.52. MS (Positive mode), m/z (%):674 ($M+H^+$+2, 21%), 672 ($M+H^+$, 59%), 627 ($M+H^+$-45, 24%), 452 ($M+H^+$-220, 100%).

BIOLOGY

Antibacterial activity:

Two gram positive bacteria,*Staphylococcus aureus*(ATCC-19433)and *Bacillus subtilis*(ATCC-23857) and two gram negative bacteria such as *Escherichia coli* (ATCC-10148)and *Pseudomonas marginalis*(MTCC-2758) were selected for screening the antibacterial activity of the newly synthesized α-minophosphonates**6(a-f)** and *N*-(substitutedphenyl)sulfonamido α-aminophosphonates**8(a-e)** using the agar well diffusion method [23]. 2 mg of the each title compound, standard drug (Nystatin) were dissolved in 2 mL of DMSO and further diluted to prepare different concentration (50, 100, 200 µg/mL) of test solutions and accurately 1 mL of these prepared samples were used for test. Centrifuged pellets of bacteria from a 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 was prepared by suspending nutrient agar 20 g in one liter of distilled water (P^H 7.0), autoclaved and cooled to 45 ^oC. Then it was seeded with 10 mL of prepared inocula to have 10^6 CFU/mL. Petri dishes were prepared by pouring 75 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 and antibacterial activity was assayed by measuring zones of inhibition in diameter around the well. The zone of inhibition of the tested solution was compared with standard. The bacterial assays were performed in triplicate and results are shown in **Table-1**.

Table-1Antibacterial activity of the newly synthesized α-minophosphonates6(a-f) and *N*-(substitutedphenyl)sulfonamido α-aminophosphonates8(a-e).

Entry	Compd	Bacterial zone of inhibition (in mm) of α-minophosphonates**6(a-f)**											
		S. aureus (ATCC-19433)			*B. subtilis* (ATCC-23857)			*E. coli* (ATCC-10148)			*P. marginalis* (MTCC-2758)		
		50 µg/ml	**100** µg/ml	**200** µg/ml	**50** µg/ml	**100** µg/ml	**200** µg/ml	**50** µg/ml	**100** µg/ml	**200** µg/ml	**50** µg/ml	**100** µg/ml	**200** µg/ml
1	**6a**	9.4	10.6	16.5	11.7	15.5	17.6	10.9	12.9	18.2	11.3	14.6	17.8
2	**6b**	10.6	14.2	17.8	10.1	12.9	18.1	10.1	13.2	17.4	11.1	14.6	19.8
3	**6c**	10.3	13.2	15.9	9.8	13.1	17.5	10.2	13.4	18.1	10.5	13.5	16.9
4	**6d**	11.0	13.1	16.5	11.5	14.2	18.0	11.0	13.5	17.5	10.8	13.5	17.0
5	**6e**	11.5	13.8	18.2	13.4	16.3	20.0	14.5	14.6	20.8	13.5	15.5	19.5
6	**6f**	10.8	11.2	16.7	11.5	13.6	17.8	13.4	15.1	19.6	12.4	15.1	17.8
Bacterial zone of inhibition (in mm) of *N*-(substitutedphenyl)sulfonamide α-aminophosphonates**8(a-e)**													
7	**8a**	13.5	16.1	21.6	14.3	16.5	20.5	15.0	17.1	20.9	14.2	17.0	21.0
8	**8b**	15.1	18.3	23.0	16.0	18.5	23.7	18.2	19.7	25.7	15.6	17.2	22.1
9	**8c**	12.5	14.3	19.4	13.6	16.4	20.1	13.9	16.5	19.4	15.0	17.3	20.1
10	**8d**	12.4	14.8	18.7	13.5	16.0	19.8	13.5	16.9	20.5	14.1	17.6	19.5
11	**8e**	12.5	14.5	19.9	13.5	16.9	21.2	15.0	17.8	20.7	15.5	19.5	22.6
12	**Std**	16	18	24	18	20	25	19	22	26	17	19	24

Std-Norfloxacin is a standard used in antibacterial activity

Antifungal bioassay

For the antifungal activity determination, 2 mg of the each title compound, standard drug were dissolved in 2 mL of DMSO and further diluted to prepare different concentration (50, 100, 200 µg/mL) of test solutions. The antifungal activity of the synthesized α-minophosphonates**6(a-f)** and *N*-(substitutedphenyl)sulfonamido α-aminophosphonates**8(a-e)** were screened against fungi,*Aspergillusniger* (MTCC-1881), *Candida albicans* (ATCC-2091)and *Aspergillus fumigates* (ATCC-9197) using agar disc-diffusion method[24]. The fungal strains were maintained on potato dextrose agar (PDA) medium (Hi-Media). A loop full of culture from the slant was inoculated into the potato dextrose broth and incubated at 37˚C for 48-72 h. Then 0.1 mL of this culture was spread on the potato dextrose agar plate and a sterile glass spreader was used for even distribution of the inoculum. Sterile discs of Whatmann No.1 filter paper of about 6 mm diameter were impregnated on the surface of the media. Blank test showed that the DMF solvent used in the preparations of the test solutions does not affect the test organisms. Different concentrations (50, 100, 200 µg/mL) of various test compounds were prepared and applied on the discs and incubated for 48-72 h at 37 ˚C. The zone of inhibition around the disc was calculated edge to edge zone of the confluent growth which corresponds to the sharpest edge of the zone and was measured in millimeters. All tests were repeated for three times and average data were taken as final result. Norfloxacin was used as a standard drug and the inhibition zones of the test compounds were compared with controls (**Table-2**).

Table-2Antifungal activity of the synthesized α-minophosphonates6(a-f) and *N*-(substitutedphenyl)sulfonamido α-aminophosphonates8(a-e).

Entry	Compd	Fungal zone of inhibition (in mm) of α-minophosphonates**6(a-f)**								
		Aspergillusniger (MTCC-1881)			*Candida albicans*(ATCC-2091)			*Aspergillus fumigates* (ATCC- 9197)		
		50 µg/ml	**100** µg/ml	**200** µg/ml	**50** µg/ml	**100** µg/ml	**200** µg/ml	**50** µg/ml	**100** µg/ml	**200** µg/ml
1	**6a**	10.5	13.8	16.0	11.0	14.5	18.5	11.2	14.5	18.1
2	**6b**	13.2	16.6	19.6	11.5	13.9	17.6	11.0	15.0	18.8
3	**6c**	10.4	14.1	16.5	10.5	14.0	17.0	10.7	13.8	17.1
4	**6d**	11.1	14.0	17.2	12.3	15.9	19.6	11.0	14.0	18.2
5	**6e**	13.5	17.4	19.5	12.3	14.8	18.5	13.0	14.9	19.2
6	**6f**	12.4	17.0	19.8	10.5	14.3	18.0	10.4	14.0	17.8
Fungal zone of inhibition (in mm) of *N*-(substitutedphenyl)sulfonamido α-aminophosphonates**8(a-e)**										
7	**8a**	13.8	17.1	19.7	13.0	15.2	19.3	12.8	16.5	20.1
8	**8b**	15.5	17.8	21.4	13.6	15.9	20.0	15.0	17.9	21.3
9	**8c**	13.6	16.8	19.5	12.0	15.3	18.0	12.6	15.1	18.9
10	**8d**	13.6	17.0	19.4	12.5	16.0	19.8	12.9	15.2	19.6
11	**8e**	13.1	16.5	19.3	12.8	15.0	19.1	13.4	14.5	19.3
12	**Std**	17	18	23	14	16	21	15	17	23

Std-Nystatin is a standard used in antifungal activity

Antioxidant activity:

DPPH radical scavenging activity [25]**:**

The 1,1-diphenyl-1-picrylhydrazyl (DPPH) has been widely used to evaluate the free radical scavenging capacity of different antioxidants. The radical in the DPPH gives a strong absorption maximum at 517 nm and the absorbance of DPPH reduces, when the radical of the DPPH becomes paired with an electron or acceptance of the hydrogen radical from the antioxidant. All the tested samples in various concentrations (25, 50, 75 and 100 µg/mL) were prepared in MeOH and the homogeneous solutions were achieved by stirring on magnetic stirrer. A 1 mL of aliquot of test sample was added to 4 mL of 0.004% (w/v) methanol solution of DPPH and the reaction mixture was vortexed for 1 min. The room temperature was recorded and kept at room temperature for 30 min in the dark to complete the reaction. The absorbance was read against blank at 517 nm. The synthetic antioxidant BHT was used as positive control. The ability of the tested samples at tested concentration to scavenge DPPH radicals were calculated suing the following equation. The experiment was carried out in triplicate and the average values are tabulated in **Table-3**.

$$\% \text{ of scavenging} = [1 - (A_{sample} - A_{sample\ blank})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control (DPPH solution without the test compound solution) and A_{sample} is the absorbance of the test sample (DPPH solution with the test compound solution) and $A_{sample\ blank}$ is the absorbance of the sample solution (the test compound solution without DPPH solution).

Table-3*In vitro* antioxidant activity of the synthesized α-minophosphonates6(a-f) and *N*-(substitutedphenyl)sulfonamido α-aminophosphonates8(a-e) by DPPH method.

Entry	Compd	The ability to scavenging the DPPH radicals by α-minophosphonates**6(a-f)**			
		25 µg/mL	**50 µg/mL**	**75 µg/mL**	**100 µg/mL**
1	**6a**	46.61 ± 1.03	57.08 ± 0.29	65.98 ± 0.64	69.98 ± 1.22
2	**6b**	49.23 ± 0.64	59.52 ± 0.36	66.28 ± 1.02	70.84 ± 0.55
3	**6c**	52.64 ± 0.72	65.40 ± 0.08	70.16 ± 0.42	79.82 ± 0.62
4	**6d**	53.58 ± 1.03	64.88 ± 0.32	71.25 ± 0.50	87.50 ± 0.10
5	**6e**	59.21 ± 0.21	69.64 ± 0.13	77.68 ± 0.28	91.23 ± 0.70
6	**6f**	52.15 ± 0.17	62.42 ± 0.42	69.37 ± 0.81	78.92 ± 0.64
The ability to scavenging the DPPH radicals by *N*-(substitutedphenyl)sulfonamido α-aminophosphonates**8(a-e)**					
7	**8a**	54.52 ± 0.36	69.36 ± 0.08	78.25 ± 0.30	88.72 ± 0.42
8	**8b**	59.80 ± 0.48	72.24 ± 0.64	80.47 ± 0.69	94.54 ± 0.12
9	**8c**	48.64 ± 0.64	62.34 ± 0.40	69.26 ± 0.46	74.19 ± 1.08
10	**8d**	57.68 ± 1.08	66.52 ± 0.34	72.13 ± 0.61	85.82 ± 0.50
11	**8e**	60.38 ± 0.18	69.90 ± 0.60	80.48 ± 0.01	95.22 ± 0.58
12	**Standard**	61.22 ± 0.02	75.74 ± 0.05	86.33± 0.02	97.65 ± 0.01
13	**Blank**	–	–	–	–

Standard-Butylatedhydroxytoluene; Balnk- Methanol; (-) Showed no scavenging activity. Values were the means of three replicates ±SD.

Determination of IC_{50} values: The half-maximal inhibitory concentrations (IC_{50}) of the tested samples were calculated by plotted a curve for concentrations of the test sample against percentage of inhibition of DPPH radicals. The IC_{50} values of all the synthesized compounds were shown in **Graph-1**.

Nitric oxide (NO) method:

Nitric oxide scavenging activity of tested samples for nitric oxide radicals (NO) was measured using slightly modified protocol of Green *et al*. and Marcocci*et al* [26]. All the tested samples in various concentrations (25, 50, 75 and 100 µg/mL) were prepared in MeOH and the homogeneous solutions were achieved by stirring on magnetic stirrer. Nitric oxide radicals (NO) were generated from 1 mL of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 and 100 µg/mL) of the test compounds and incubated for 150 min at 25^{o}C. 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediaminedihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Butylated hydroxyl toluene was used as positive control. The ability to scavenge the NO radicals was calculated by the following equation.

$$\% \text{ of scavenging} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (all reagents solution without the test compound solution), A_{sample} is the absorbance of the test compound (all the reagents solution with the test compound solution). The experiment was carried out in triplicate and the results are presented in **Table-4**.

Graph-1 Antioxidant IC_{50} values of the synthesized α-minophosphonates6(a-f) and *N*-(substitutedphenyl)sulfonamide α-aminophosphonates8(a-e) by DPPH method.

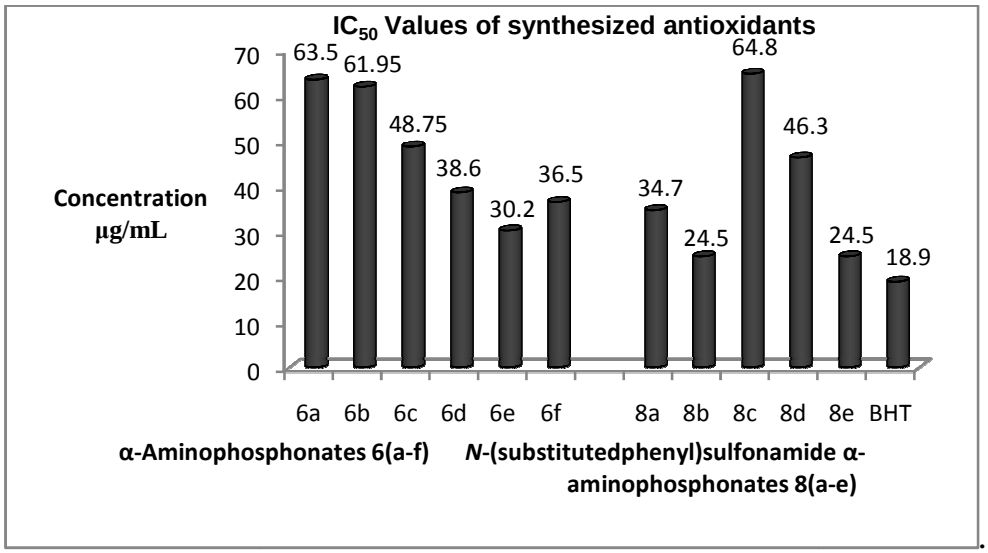


Table-4*In vitro* antioxidant activity of the newly synthesized α-minophosphonates6(a-f) and *N*-(substitutedphenyl)sulfonamido α-aminophosphonates8(a-e) by NO method.

Entry	Compd	The ability to scavenging the NO radicals by α-minophosphonates**6(a-f)**			
		25 µg/mL	**50 µg/mL**	**75 µg/mL**	**100 µg/mL**
1	**6a**	40.24 ± 0.56	48.30 ± 0.20	53.08 ± 0.22	60.06 ± 0.84
2	**6b**	43.53 ± 0.31	48.88 ± 0.28	56.02 ± 0.48	64.20 ± 0.30
3	**6c**	43.64 ± 1.06	50.62 ± 1.08	59.26 ± 0.40	65.12 ± 0.63
4	**6d**	46.84 ± 0.10	55.61 ± 0.29	71.30 ± 0.54	77.85 ± 0.49
5	**6e**	48.86 ± 0.16	57.32 ± 0.52	74.72 ± 0.18	89.78 ± 0.26
6	**6f**	45.50 ± 0.25	58.08 ± 0.98	69.26 ± 0.28	82.59 ± 0.61
The ability to scavenging the DPPH radicals by *N*-(substitutedphenyl)sulfonamido α-aminophosphonates**8(a-e)**					
7	**8a**	47.11 ± 0.20	57.92 ± 0.22	72.18 ± 0.52	85.53 ± 0.11
8	**8b**	50.20 ± 0.28	60.45 ± 0.40	75.81 ± 0.33	91.36 ± 0.24
9	**8c**	38.94 ± 0.10	46.72 ± 0.38	58.37 ± 0.21	67.82 ± 1.10
10	**8d**	44.56 ± 1.12	52.03 ± 0.35	64.86 ± 0.40	78.59 ± 0.61
11	**8e**	50.28 ± 0.28	61.72 ± 0.60	75.56 ± 0.92	90.14 ± 0.76
12	**Standard**	54.68 ± 0.32	68.47 ± 0.01	79.24 ± 0.08	95.38 ± 0.06
13	**Blank**	–	–	–	–

Standard-Butylatedhydroxytoluene; Balnk- Methanol; (-) Showed no scavenging activity. Values were the means of three replicates ±SD.

Hydrogen Peroxide scavenging method

The ability of hydrogen peroxide scavenging activity of the tested samples was determined according to the protocol of Nabavi*et al* [27]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Different concentrations (25, 50, 75 and 100 µg/mL) in MeOH were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the tested and standard compounds was calculated as follows.

$$\% \text{ of scavenging} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where 'A $_{control}$' was the absorbance of the standard and 'A $_{sample}$' was the absorbance in the presence of the sample and standard. The experiment was carried out in triplicate and the results are presented in **Table-5**.

Table-5*In vitro* antioxidant activity of the synthesized α-minophosphonates6(a-f) and *N*-(substitutedphenyl)sulfonamido α-aminophosphonates8(a-e) by H_2O_2 method.

Entry	Compd	The percentage of hydrogen peroxide scavenging by α-minophosphonates**6(a-f)**			
		25 µg/mL	**50 µg/mL**	**75 µg/mL**	**100 µg/mL**
1	**6a**	36.23 ± 0.91	42.68 ± 0.20	48.75 ± 0.30	52.92 ±0.54
2	**6b**	40.64 ± 1.04	49.54 ± 0.42	56.98 ± 0.06	64.86 ± 0.42
3	**6c**	43.20 ± 0.24	57.21 ± 0.69	64.08 ± 0.72	70.32 ± 0.30
4	**6d**	47.08 ± 1.23	61.32 ± 0.05	68.65 ± 0.15	75.01 ± 0.43
5	**6e**	49.96 ± 0.19	62.28 ± 0.18	70.72 ± 0.56	85.48 ± 0.44
6	**6f**	47.33 ± 0.53	59.64 ± 1.52	67.51 ± 0.31	78.41 ± 0.30
The percentage of hydrogen peroxide scavenging by *N*-(substitutedphenyl)sulfonamido α-aminophosphonates**8(a-e)**					
7	**8a**	42.54 ± 0.88	55.38 ± 0.38	69.27 ± 0.50	76.42 ± 0.18
8	**8b**	50.20 ± 1.14	61.02 ± 0.62	72.39 ± 0.23	84.18 ± 0.26
9	**8c**	34.82 ± 0.76	46.35 ± 0.55	56.64 ± 0.34	69.15 ± 0.20
10	**8d**	45.69 ± 0.12	57.42 ± 0.40	70.14 ± 0.72	79.85 ± 0.61
11	**8e**	49.81 ± 0.93	61.24 ± 0.88	72.45 ± 0.24	83.21 ± 0.34
12	**Standard**	54.27 ± 0.05	64.68 ± 0.11	79.28 ± 0.02	88.34 ± 0.01
13	**Blank**	–	–	–	–

Standard-Ascorbic acid; Balnk- Methanol; (-) Showed no scavenging activity. Values were the means of three replicates ±SD.

RESULTS AND DISCUSSION

The ubiquitous biological values of the α-aminophosphonates have stimulated increasing interest for the development of novel organophosphorus compounds. In our continuous research on the synthesis of novel biologically active phosphorus molecules [28], we have developed an efficient green approach for the synthesis of α-aminophosphonates of 2-aminothiophene and also synthesized novel *N*-substitutedphenyl sulfonamide α-aminophosphonateswhich are depicted in **Scheme-1** and **Scheme-2**respectively. Moreover, to the best of our knowledge and literature survey revealed that thiswould be the first report on the construction and antimicrobial and antioxidant activities of novel *N*-substitutedphenyl sulfonamide α-aminophosphonates.

At first, we synthesized an amine, ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate **(3)** by Gewalds reaction [29] by reacting ethyl acetoacetate, malononitrile and sulfur powder in EtOHusing dimethyl piperazine as base at ambient temperature.Synthesized amine **3**, *m*-nitro benzaldehyde **(4a)** and diethylphosphite **(5)** were selected as models for the optimization of the reaction conditions to synthesize α-aminophosphonates. The effect of the reaction was monitored in different catalysts (10 mol%) in THF to afford compound **6a** (**Table-6, entry 1-5**). Remarkably, the high yield of compound **6a** was observed at the reaction conditions monitored in silica supported $ZnCl_2$ (**Table-6, entry 5**).This result attracted to investigate further the best reaction conditions, the same reaction was conducted in different solvents and solvent-free conditions (**Table-7, entry 1-7**) and the optimum yield of the desired product was observed in solvent-free condition (**Table-7, entry 5-7**) as compared with solvent conditions (8-15%).The effect of the amount of the catalyst was scrutinized by altering the amount of the catalyst under solvent-free conditions (**Table-8, entry 1-9**), the high yield was observed at the 15 mol% amount of the catalyst (**Table-8, entry 4**). The reusability of $SiO_2/ZnCl_2$ catalyst was also examined. After each run, the product was filtered and the residue of catalyst was washed with $CHCl_3$ to remove stains from the catalyst surface and reused up to five cycles forthe synthesis of compound **6a** and re-examined these reactions (**Table-8, entry 6-9**).It was found that $SiO_2/ZnCl_2$ is inexpensive catalyst and could effectively catalyze this reaction under solvent-free conditions to afford the desired products in high yields.After optimization of the reaction conditions, a variety of aromatic aldehydes bearing electron with drawing and electron donating groups are altered to assess the generality of the reaction and the desired α-aminophosphonateproducts were obtained in high yields and the results are summarized in **Table-9 (entry 1-6)**. The antimicrobial and antioxidant activities of the synthesized α-aminophosphonates were evaluated (**Table-1**, **Table-2** and **Table-3**, **Table-4**, **Table-5, entry 1-6**). The biological data revealed that compound **6e** exhibited good antimicrobial activity against tested pathogens as well as good antioxidant activity in all the tested DPPH, NO, H_2O_2 methods.

Table-6Optimization of theKabachnik-Fields reaction to synthesize α-aminophosphonates[a].

Entry	Catalyst	Time (h)	Yield (%)[b]
1	No catalyst	16	52.3
2	$CuCl_2$ (10 mol%)	10	61.8
3	$AlCl_3$ (10 mol%)	8	73.2
4	$ZnCl_2$ (10 mol%)	6	79.3
5	SiO_2-$ZnCl_2$ (10 mmol)	4	86.4

*[a]The reaction was performed using ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate **(3)** (1 mmol), m-nitro benzaldehyde**(4a)** (1 mmol) and diethylphosphite**(5)** (1.2 mmol) in THF at 35 °C.; [b]Isolated yields.*

Table-7The solvent effect on the Kabachnik-Fields reaction catalyzed by SiO_2-$ZnCl_2$ (10 mol%) catalyst[a].

Entry	Solvent	Time (h)	Yield (%)[b]
1	THF	4	86.4
2	Toluene	6	72.7
3	ACN	5	82.3
4	EtOH	5	84.1
5	Solvent-free	3	88.2
6	Solvent-free[c]	3	89.4
7	Solvent-free[d]	3	89.7

*[a]The reaction was performed using ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate **(3)** (1 mmol), m-nitro benzaldehyde**(4a)** (1 mmol) and diethylphosphite**(5)** (1.2 mmol).*
[b]Isolated yields.; [c]The reaction progressed at 40 °C.; [d]The reaction progressed at 45 °C.

Table-8The effect of the amount of the catalyst, SiO_2-$ZnCl_2$ to promote the Kabachnik-Fields reaction[a].

Entry	Amount of SiO_2-$ZnCl_2$	Time (h)	Yield (%)[b]
1	7.5 mol%	5	79.2
2	10.0 mol%	3	89.4
3	12.5 mol%	3	91.3
4	15.0 mol%	3	93.4
5	17.5 mol%	3	93.5
6[c]	15.0 mol%	3	92.0
7[d]	15.0 mol%	3	91.8
8[e]	15.0 mol%	3	91.1
9[f]	15.0 mol%	3.5	89.2

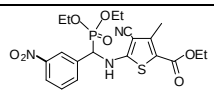
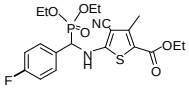
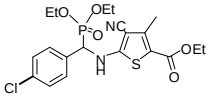
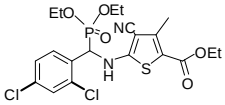
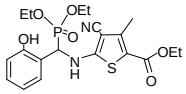
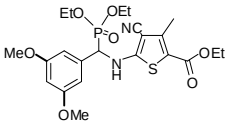
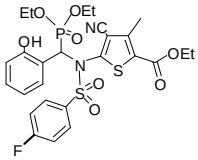
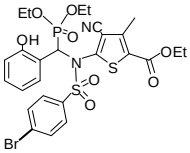
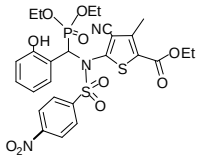
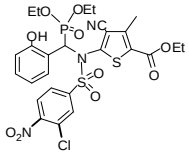
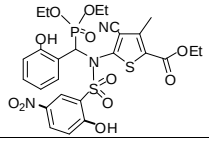
*[a]The reaction was performed using ethyl 5-amino-4-cyano-3-methylthiophene-2-carboxylate **(3)** (1 mmol), m-nitro benzaldehyde**(4a)** (1 mmol) and diethylphosphite**(5)** (1.2 mmol) at 40 °C.*
[b]Isolated yields; [c]The re-usability of the catalyst, SiO_2-$ZnCl_2$ in 2nd run; [d]The re-usability of the catalyst, SiO_2-$ZnCl_2$ in 3rd run; [e]The re-usability of the catalyst, SiO_2-$ZnCl_2$ in 4th run; [f]The re-usability of the catalyst, SiO_2-$ZnCl_2$ in 5th run.

Further, with the hope to enhance the activity, the active newly synthesized α-aminophosphonate derivative**6e** was utilized as precursor to synthesize a new class of phosphonated *N*-substitutedphenyl sulfonamide derivatives. The –NH nucleophile of α-aminophosphonate**6e** underwent nucleophilic substitution reaction with bio-active functionalized phenylsulfonyl chlorides **7(a-e)** by refluxing in THF using 1.2 equivalent of 1,4-dimethyl piperazine (DMP) as a base to afford desired titled compounds **8(a-e)** in moderate to high yields (**Table-9, entry 7-11**). The desired title compounds were screened for their antimicrobial and antioxidant activities (**Table-1**, **Table-2** and **Table-3**, **Table-4**, **Table-5 entry 7-11**). Interestingly, there is significant enhancement in antimicrobial activity and no enhancement in antioxidant activity of the title compounds **8(a-e)**when compared with α-aminophosphonate**6e**.

Structures of all the newly synthesized compounds **6(a-f)**and **8(a-e)** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, mass and elemental analysis data. IR spectra of compounds **6(a-f)** exhibited absorption bands at 3215-3380 indicating the presence of amine –N-H, the stretching frequencies at 1220-1280 and 1005-1070 confirmed the presence of –P=O and –P-O-$C_{(alipha)}$- functionalities respectively. The characteristic absorption bands in IR spectra at 1340-1370 and 1105-1140 were assigned to –SO_2 functionality in**8(a-e)**. In ^{1}H NMR spectra, the peaks at 4.18-4.60 ppm and 5.35-5.93 ppm are assigned to –P-CH- and –C-NH- functionalities in α-aminophosphonates **6(a-f)** and the disappearance of the peak at 5.35-5.93 ppm of –C-NH-, small downfield frequency enhancement from 4.18-4.60 ppm to 5.60-5.90 of –P-CH- confirmed the formation of the *N*-sulfonamide products **8(a-e)** by substituting the sulfonayl chlorides at –NH of α-aminophosphonate**6e**. All the phenyl protons showed peaks as multiplets/triplets/doublets/ singlets based on structures in the region of 6.80-8.97 ppm. ^{31}P NMR spectra exhibited the peaks at expected region 19.8-26.4 ppm for the synthesized compounds. The corresponding 'C' chemical shifts of the products in ^{13}C NMR spectra, the molecular ions and fragmentation peaks (daughter ions) in mass spectra and

elemental analytical data gave further evidence for structural elucidation of the newly synthesized products**6(a-f)** and **8(a-e)**.

Table-9 Physical data of the synthesized α-minophosphonates6(a-f) and *N*-(substitutedphenyl) sulfonamido α-aminophosphonates8(a-e).

Entry	Compd	Product	Conventional conditions		M. P (°C)
			Time (h)	Yield (%)	
1	**6a**		3	93.4	145-146
2	**6b**		4	90.8	157-159
3	**6c**		4	89.0	136-138
4	**6d**		4.5	88.5	131-133
5	**6e**		4	89.6	182-183
6	**6f**		4.5	89.7	176-178
7	**8a**		3	82.6	152-153
8	**8b**		4	84.7	174-176
9	**8c**		3	89.3	123-125
10	**8d**		3	87.2	145-147
11	**8e**		3	84.8	167-169

Synthesized compounds, α-aminophosphonates **6(a-f)** and*N*-substitutedphenyl sulfonamide α-aminophosphonates **8(a-e)** were screened for their antibacterial activity against two gram positive bacteria such as *Staphylococcus aureus* (ATCC-19433),*Bacillus subtilis* (ATCC-23857) and two gram negative bacteria such as *Escherichia coli* (ATCC-10148),*Pseudomonas marginalis* (MTCC-2758) using agar well diffusionmethod[23]and fungi, *Aspergillus*

niger (MTCC-1881), *Candida albicans* (ATCC-2091)and *Aspergillus fumigates* (ATCC-9197) using agar disc diffusionmethod [24]. Among the phosphonates**6(a-f)**, the compounds **6e** and **6b** showed promising antimicrobial activity against all the tested pathogens, compound **6f** exhibited high inhibition against *E. coli*, the compound **6e** against *A. niger* and the compound **6d** against *C. albicans*exhibited good inhibitory activity. In the sulfonamide derivatives**8(a-e)**, the compounds **8a**, **8b** and **8e** showed significant antimicrobial activity and the compound **8d** showed good activity against *A. fumigates* (**see Table-1 and Table-2**).

Antioxidant activity was examined using DPPH [25], NO [26] and H_2O_2 [27] methods, the results are tabulated in **Table-3**, **Table-4** and **Table-5** respectively and IC_{50} values were also determined for all the tested samples (**Graph-1**). All the tested samples showed good to moderate antioxidant activity. Among the compounds **6(a-f)**, the compound**6e** exhibited good activity in all the tested methods.In the compounds **8(a-e)**, the compounds **8b** and **8e** showed promising antioxidant activity when compared with α-aminophosphonates. The functionalities like –OH in compound **6e**, -Br, -OH in the compound **8b** and two –OH groups in the compound **8e** might be the cause to exhibit significant antioxidant activity. In overall biological data, noteworthy enhancement in antimicrobial activity and no significant enhancements in antioxidant activity were observed in *N*-substitutedphenylsulfonamido α-aminophosphonates**8(a-e)** when compared with active α-aminophosphonate**6e**.

CONCLUSION

In conclusion, we have synthesized a series of novel α-aminophosphonates**6(a-f)** of multisubstituted 2-aminothiophene under solvent-free conditions using a heterogeneous catalyst, $SiO_2/ZnCl_2$, which is an inexpensive and reusable catalyst. Further, novel *N*-substitutedphenyl sulfonamide α-aminophosphonates**8(a-e)**were prepared from biologicallyactiveα-aminophosphonate **6e**.All the newly synthesized compounds were screened for their*in vitro* antimicrobial and antioxidant activities. The biological data revealed that α-aminophosphonates are effectively worked as antioxidants and *N*-substitutedphenyl sulfonamide α-aminophosphonates are meritoriously worked as antimicrobial agents against tested microorganisms. In overall, remarkable enhancement in antimicrobial activity and no significant enhancements in antioxidant activity were observed in *N*-substitutedphenylsulfonamido α-aminophosphonates**8(a-e)** when compared with α-aminophosphonates**6(a-f)**. The reported chemical motifs in this study might provide a conceivable prospect for developing of new medicinal agents with promising pharmacological properties and good contribution to the phosphorus and sulfur chemistry.

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