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Synthesis, characterisation and antimicrobial-activity of 3-thio-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amino acid esters

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Abstract: A new series of 3-thio-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amino acid esters (4a-k) were synthesized by treating different amino acid ester hydrochlorides (3a-k) with phosphorus monochloride intermediate (2) which was previously formed in situ from 1,2-phenylenedimethanol (1) and thiophosphoryl chloride in the presence of triethylamine in dry tetrahydrofuran (THF) at 0-5 ºC to room temperature. The structures of the title compounds (4a-k) were established by analytical, IR, NMR (1H, 13C and 31P) and mass spectra, and they have been screened for their antimicrobial activity. They exhibited significant antibacterial, and antifungal activity.

Keywords: Phosphoramides; 1,2-phenylenedimethanol; amino acid hydrochloride; antibacterial activity; antifungal activity.

1. Introduction

Phosphoramides substituted with an amino acid ester is an important class of rationally designed therapeutics especially with antineoplastic properties.[1] Thiophosphoramidate derivatives of the anti-HIV nucleosides were also reported to act as membrane soluble prodrugs of the bioactive free nucleotides.[2]

In addition, phosphocin/ phosphepine and its related derivatives containing this group represent an important class of pesticides, antibiotics, herbicides, and antiviral agents.[3-7] Some of them are well known for their insecticidal activities,[8] fungicidal properties,[9] and are known to degrade hydrolytically and enzymatically to non-toxic residues. Further hydrolysis of the exocyclic P–N bond may release products of limited toxicity to the biosystem.[10] Recently the first thiophosphonate functional group compounds as pincer ligands were synthesized and characterized followed by preparation of stable co-ordination complexes of palladium and silver metals.[11] These results

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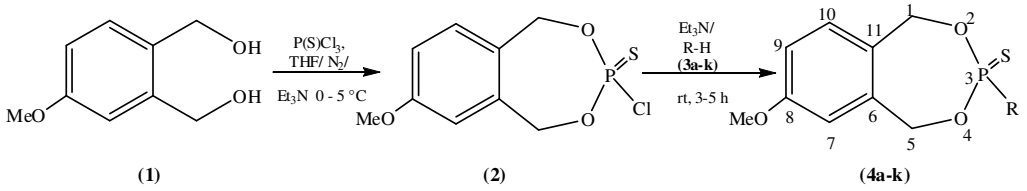
illustrated that the thiophosphonate functional group interacts as lateral binding sites to the stable metal complexes.

In view of this we have synthesized and accomplished a new class of heterocyclic compounds and tested for their efficiency in inhibiting growth of bacteria, and fungi.

2. Results and discussion

Synthesis of 3-thio-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amino acid esters (**4a-k**) were accomplished in a one-pot process. The synthetic route (**Scheme 1**) involves the cyclization of equimolar quantities of 1,2-phenylenedimethanol (**1**) with thiophosphoryl chloride in the presence of triethylamine in dry tetrahydrofuran (THF) at 0-5 ºC temperature to afford the corresponding monochloride (**2**) *in situly*. On subsequent reaction of this unstable **2** with different amino acid ester hydrochlorides (**3a-k**) in the presence of triethylamine in dry THF at room temperature with stirring for 3-5 h to obtain **4a-k**.



Compound	R	Compound	R
4a	$-HN-CH_2-CO_2CH_3$	**4g**	$-NH-CH(COOCH_3)-CH_2$-(imidazole)
4b	$-HN-CH(CH_3)-CO_2CH_3$	**4h**	$-NH-CH(COOCH_3)-CH_2-C_6H_5$
4c	$-HN-CH(CH_3)-CO_2C_2H_5$	**4i**	$-NH-CH(COOC_2H_5)-C_6H_5$
4d	$-HN-CH(CH(CH_3)_2)-CO_2CH_3$	**4j**	$-HN-CH(CO_2CH_3)$-(indole)
4e	$-HN-CH(CO_2CH_3)-CH_2-CH(CH_3)_2$	**4k**	N-(ethyl indole-2-carboxylate)
4f	$-HN-CH(CO_2Et)-CH_2-CH_2-CH_3$		

Scheme 1. Synthetic of benzodioxaphosphepin-3-amino Acid Esters (**4a-k**)

3-thio-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amino acid esters

Product yields and elemental analysis, IR, ^{1}H-, ^{31}P- and ^{13}C-NMR data of **4a–k** are given and the data agreed with the proposed chemical structures. Compounds **4a-k** exhibited characteristic IR stretching frequencies in the regions 3421-3439, 1729-1751 and 779-798 cm^{-1} for N-H, C=O and P=S respectively.[12]

In ^{1}H-NMR spectra,[12] the aromatic protons in the compounds **4a-k** gave as multiplet in the region δ 6.94-7.91. The four benzylic proton carbons those are directly attached to oxygen atom resonated as a two distinguish doublet of doublets at δ 5.66-5.81 and δ 4.69-4.93 indicating their non-equivalence in the seven membered chair conformation of the benzodioxaphosphepin ring system (**Scheme-2**). The N-H proton appeared as a broad singlet at δ 8.37-8.75. All the remaining protons are resonated at respective region. The ^{13}C-NMR spectral data for **4a, b, d, e, f, & 4i** are given in the experimental section.[12] The ^{31}P-NMR resonates as a singlet at δ 60.6-63.2 (P=S) for the title compounds.[13] The mass spectra of compounds **4a, b, d & 2i** showed their respective molecular ion peaks in the expected m/z mass values.

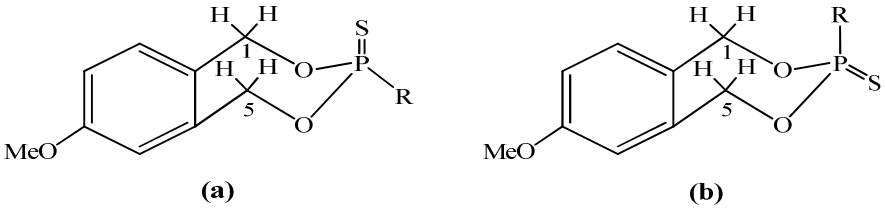


Figure -1: Chair configurations of benzodioxaphosphepin-3-Amino Acid Esters (4a-k)

3. Conclusion

In conclusion, we reported an efficient easy process to synthesis biologically active benzodioxaphosphepin derivatives. The method is has short reaction time, clean reaction profile, simple experimental and workup procedures for the synthesis of (**4a-k**) compounds.

4. Experimental

The melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. The IR spectra (ν_{max}, cm^{-1}) were recorded as KBr pellets on a Perkin Elmer 1000 unit. The ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on a Varian AMX 400 MHz NMR spectrometer operating at 400 MHz for ^{1}H-, 100.57 MHz for ^{13}C- and 161.7 MHz for ^{31}P-NMR. All the compounds were dissolved in DMSO-d_6 and chemical shifts were referenced to TMS (^{1}H and ^{13}C) and 85% H_3PO_4 (^{31}P). Microanalyses data were obtained from the Central Drug Research Institute (CDRI), Lucknow, India.

4.1. General Procedure for synthesis of methyl [(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl)amino]acetate (4a-k):

Synthesis of 3-thio-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amino acid esters (**4a-k**) are accomplished in a single step process as below.

The synthetic route (**Scheme-1**) involves the cyclization of 1,2-phenylenedimethanol (**1**, 1 mmol) with thiophosphoryl chloride (1 mmol) afforded the corresponding unstable cyclic monochloride (**2**) *in situly* in the presence of triethylamine (2 mmol) in dry THF (20 mL) at 0 ºC to room temperature. Subsequent reaction of the unstable monochloride (**2**) with different amino acid ester hydrochlorides (**3a-k**, 1mmol) in triethylamine (1 mmol) in dry THF (10 mL) at room temperature with stirring for 3-5 h gave **4a-k**

respectively. Progress of the reaction was monitored by TLC analysis. The crude products obtained as residues after removing the solvent by rota-evaporator were purified by repeatedly washing with water to remove any residual triethylamine hydrochloride and then with cold methanol to remove the unreacted starting materials and other impurities. The crude title compounds (**4a-k**) were further purified by flash chromatography on silica gel, using hexane-ethyl acetate (8:2) as eluent and were obtained in high yields.

Methyl[(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl)amino]acetate (4a). Yield 68%; mp 154-156 °C; IR (KBr) (ν_{max} cm^{-1}): 3427 (N-H), 1730 (C=O), 798 (P=S); ^{1}HNMR (δ ppm): 8.66 (brs, 1H, NH), 7.03-7.56 (m, 3H, Ar-H), 5.72 (dd, 2H, $^3J_{HP}$ = 20.9 Hz, and $^2J_{HH}$ = 13.6 Hz, 1,5-H), 4.93 (dd, 2H, $^3J_{HP}$ = 20.9 Hz and $^2J_{HH}$ = 13.6 Hz, 1,5-H), 3.58 (m, 2H, NCH_2), 3.82 (s, 3H, Ar-OCH_3), 3.67 (s, 3H, -OCH_3); ^{13}CNMR (δ ppm): 170.5 (CO), 158.3 (C-8), 138.2 (C-6), 134.5 (C-11), 133.8 (C-10), 126.0 (C-9), 109.7 (C-7), 68.9 (C-1 & C-5), 53.2 (Ar-OCH_3), 52.6 (OCH_3), 43.4 (NCH_2); ^{31}PNMR (δ ppm): 61.3 ppm; MS (*m/z*): 317 ($M^{+\bullet}$, 58%). *Anal*. Calcd for: $C_{12}H_{16}NO_5PS$: C, 45.42; H, 5.08; N, 4.41. Found C, 45.35; H, 5.01; N, 4.35.

Methyl2-[(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl) amino]proanoate (4b). Yield 69%; mp 123-124 °C. IR (KBr) (ν_{max} cm^{-1}): 3439 (N-H), 1740 (C=O), 783 (P=S); ^{1}HNMR (δ ppm): 8.67 (brs, 1H, NH), 7.04-7.39 (m, 3H, Ar-H), 5.69 (dd, 2H, $^3J_{HP}$ = 21.2 Hz and $^2J_{HH}$ = 13.8 Hz, 1,5-H), 4.82 (dd, 2H, $^3J_{HP}$ = 21.2 Hz and $^2J_{HH}$ = 13.8 Hz, 1,5-H), 3.47(m, 1H, NCH), 3.81 (s, 3H, Ar-OCH_3), 3.66 (s, 3H, -OCH_3), 1.46 (d, 3H, $^3J_{HH}$ = 6.8 Hz, -CH_3); ^{13}CNMR (δ ppm): 169.4 (CO), 158.6 (C-8), 138.4 (C-6), 131.4 (C-11), 127.1 (C-9), 123.5 (C-10), 109.2 (C-7), 68.8 (C-1 & C-5), 52.7 (Ar-OCH_3), 51.3 (-OCH_3), 44.5 (NCH), 21.6 (-CH_3); ^{31}PNMR (δ ppm): 63.2 ppm; MS (*m/z*): 331 ($M^{+\bullet}$, 25%). *Anal*. Calcd for: $C_{13}H_{18}NO_5PS$: C, 47.13; H, 5.48; N, 4.23. Found C, 47.04; H, 5.42; N, 4.18.

Ethyl 2-[(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl) amino]propanoate (4c). Yield 70%; mp 120-122 °C. IR (KBr) (ν_{max} cm^{-1}): 3430 (N-H), 1739 (C=O), 785 (P=S); ^{1}HNMR (δ ppm): 8.68 (brs, 1H, NH), 7.01-7.35 (m, 3H, Ar-H), 5.70 (dd, 2H, $^3J_{HP}$ = 21.2 Hz and $^2J_{HH}$ = 13.8 Hz, 1,5-H), 4.83 (dd, 2H, $^3J_{HP}$ = 21.2 and $^2J_{HH}$ = 13.8 Hz, 1,5-H), 3.45 (m, 1H, -NCH), 4.15 (q, 2H, $^3J_{HH}$ = 6.1 Hz, -OCH_2), 3.78 (s, 3H, Ar-OCH_3), 1.28 (t, 3H, $^3J_{HH}$ = 6.1 Hz, -CH_3), 1.72 (d, 3H, $^3J_{HH}$ = 2.8, -CH_3); ^{31}PNMR (δ ppm): 62.9 ppm; *Anal*. Calcd for $C_{14}H_{20}NO_5PS$: C, 48.69; H, 5.84; N, 4.06, Found C, 48.60; H, 5.78; N, 4.01.

Methyl 2-[(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl)amino]-3-methylbutanoate (4d). Yield 66%; mp 160-162 °C. IR (KBr) (ν_{max} cm^{-1}): 3432 (N-H), 1732 (C=O), 779 (P=S); ^{1}HNMR (δ ppm): 8.48 (brs, 1H, NH), 7.12-7.41 (m, 3H, Ar-H), 5.81 (dd, 2H, $^3J_{HP}$ = 19.9 Hz and $^2J_{HH}$ = 13.9 Hz, 1,5-H), 4.69 (dd, 2H, $^3J_{HP}$ = 19.9 Hz and $^2J_{HH}$ = 13.9 Hz, 1,5-H), 3.80 (s, 3H, Ar-OCH_3), 3.62 (3H, s, -OCH_3), 3.45 (m, 1H, -NCH), 1.34 (m, 1H, -CH), 1.01 (d, 6H, $^3J_{HH}$ = 5.8 Hz, 2×CH_3); ^{13}CNMR (δ ppm): 169.5 (CO), 158.8 (C-8), 138.8 (C-6), 131.4 (C-11), 129.2 (C-7), 127.4 (C-9), 123.1 (C-10), 68.9 (C-1 & C-5), 52.2 (Ar-OCH_3), 51.5 (-OCH_3), 45.3 (C-6´), 44.8 (C-5´), 22.8 (C-7´ & C-7´´); ^{31}PNMR (δ ppm): 62.8 ppm; MS (*m/z*): 359 ($M^{+\bullet}$, 34%). *Anal*. Calcd for: $C_{15}H_{22}NO_5PS$: C, 50.13; H, 6.17; N, 3.90. Found C, 50.05; H, 6.11; N, 3.84.

Methyl 2-[(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl) amino] pentanoate (4e). Yield 68%; mp 119-121 °C. IR (KBr) (ν_{max} cm^{-1}): 3421 (N-H), 1742 (C=O), 790 (P=S); ^{1}HNMR (δ ppm): 8.49 (brs, 1H, NH), 7.10-7.33 (m, 3H, Ar-H), 5.66 (dd, 2H, $^3J_{HP}$ = 20.4 Hz and $^2J_{HH}$ = 13.4 Hz, 1,5-H), 4.75 (dd, 2H, $^3J_{HP}$ = 20.4 Hz and $^2J_{HH}$ = 13.4 Hz, 1,5-H), 3.79 (s, 3H, Ar-OCH_3), 3.58 (s, 3H, -OCH_3), 3.45 (m, 1H, NCH), 1.90 (m, 2H, CH_2), 1.33 (m, 2H, -CH_2), 1.12 (t, 3H, $^3J_{HH}$ = 6.3 Hz, -CH_3); ^{13}CNMR (δ ppm): 168.4 (CO), 158.2 (C-8), 138.4 (C-6), 132.2 (C-11), 130.1 (C-7), 128.1 (C-9), 123.5 (C-10), 69.1 (C-1 & C-5), 52.6 (Ar-OCH_3), 51.8 (-OCH_3), 38.9 (C-6´), 44.7 (C-5´), 19.2 (C-7´); ^{31}PNMR (δ ppm): 61.2 ppm; *Anal*. Calcd for $C_{15}H_{22}NO_5PS$: C, 50.13.; H, 6.17; N, 3.90, Found C, 50.04; H, 6.12; N, 3.84.

Ethyl 2-[(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl) amino] pentanoate (4f). Yield 69%; mp 123-124 °C. IR (KBr) (ν_{max} cm^{-1}): 3422 (N-H), 1751 (C=O), 798 (P=S); ^{1}HNMR (δ ppm): 8.37 (brs, 1H, NH), 7.11-7.32 (m, 3H, Ar-H), 5.68 (dd, 2H, $^{3}J_{HP}$ = 20.4 Hz and $^{2}J_{HH}$ = 13.4 Hz, 1,5-H), 4.79 (dd, 2H, $^{3}J_{HP}$ = 20.4 Hz and $^{2}J_{HH}$ = 13.4 Hz, 1.5-H), 3.45 (m, 1H, NCH), 4.13 (2H, q, $^{3}J_{HH}$ = 6.1 Hz, -OCH_2), 3.80 (s, 3H, Ar-OCH_3), 1.25 (t, 3H, $^{3}J_{HH}$ = 6.1 Hz, -CH_3), 1.90 (m, 2H, -CH_2), 1.33 (m, 2H, -CH_2), 1.12 (t, 3H, $^{3}J_{HH}$ = 6.3 Hz, -CH_3); ^{13}CNMR (δ ppm): 168.5 (CO), 158.5 (C-8), 138.5 (C-6), 132.4 (C-11), 130.3 (C-7), 128.5 (C-9), 123.1 (C-10), 69.2 (C-1 & C-5), 52.5 (Ar-OCH_3), 56.5 (-OCH_2), 38.6 (C-6´), 44.8 (C-5´), 19.7 (C-7´), 13.8 (-CH_3); ^{31}PNMR (δ ppm): 61.8 ppm; *Anal*. Calcd for: $C_{16}H_{24}NO_5PS$: C, 51.46; H, 6.48; N, 3.75. Found C, 51.37; H, 6.40; N, 3.68.

Methyl 3-(1H-imidazol-4-yl)-2-[(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl)amino]propanoate (4g). Yield 68%; mp 118-120 °C. IR (KBr) (ν_{max} cm^{-1}): 3430 (N-H), 1740 (C=O), 789 (P=S); ^{1}HNMR (δ ppm): 8.56-8.72 (brs, 2H, NH), 6.94-7.71 (m, 5H, Ar-H), 5.66 (dd, 2H, $^{3}J_{HP}$ = 20.4 Hz and $^{2}J_{HH}$ = 13.4 Hz, 1,5-H), 4.78 (dd, 2H, $^{3}J_{HP}$ = 20.4 Hz and $^{2}J_{HH}$ = 13.4 Hz, 1,5-H), 3.81 (s, 3H, Ar-OCH_3), 3.58 (s, 3H, -OCH_3), 3.55 (m, 1H, NCH), 2.13 (d, 2H, $^{3}J_{HH}$ = 6.5 Hz, -CH_2); ^{31}PNMR (δ ppm): 61.5 ppm; *Anal*. Calcd for $C_{16}H_{20}N_3O_5PS$: C, 48.36; H, 5.07; N, 10.57, Found C, 48.29; H, 5.01; N, 10.51.

Methyl2-[(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl)amino]-3-phenylpropanoate (4h). Yield 70%; mp 170-172 °C. IR (KBr) (ν_{max} cm^{-1}): 3435 (N-H), 1744 (C=O), 782 (P=S); ^{1}HNMR (δ ppm): 8.60 (brs, 1H, NH), 7.45-7.91 (m, 8H, Ar-H), 5.75 (dd, 2H, $^{3}J_{HP}$ = 21.4 Hz and $^{2}J_{HH}$ = 14.2 Hz, 1,5-H), 4.90 (dd, 2H, $^{3}J_{HP}$ = 21.4 Hz and $^{2}J_{HH}$ = 14.2 Hz, 1,5-H), 3.82 (s, 3H, Ar-OCH_3), 3.63 (s, 3H, -OCH_3), 3.37 (m, 1H, -NCH), 3.08 (d, 2H, $^{3}J_{HH}$ = 6.4 Hz, -CH_2-Ar); ^{31}PNMR (δ ppm): 62.1 ppm; *Anal*. Calcd for $C_{19}H_{22}NO_5PS$: C, 56.01; H, 5.44; N, 3.44, Found C, 55.94; H, 5.38; N, 3.38.

Ethyl2-[(7-methoxy-3-sulfido-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl)amino]-3-phenylpropanoate (4i). Yield 69%; mp 172-173 °C. IR (KBr) (ν_{max} cm^{-1}): 3435 (N-H), 1743 (C=O), 785 (P=S); ^{1}HNMR (δ ppm): 8.60 (brs, 1H, NH), 7.46-7.91 (m, 8H, Ar-H), 5.75 (dd, 2H, $^{3}J_{HP}$ = 21.4 Hz and $^{2}J_{HH}$ = 14.2 Hz, 1,5-H), 4.90 (dd, 2H, $^{3}J_{HP}$ = 21.4 Hz and $^{2}J_{HH}$ = 14.2 Hz, 1,5-H), 4.14 (q, 2H, $^{3}J_{HH}$ = 6.1 Hz, -OCH_2), 3.81 (s, 3H, Ar-OCH_3), 3.38 (m, 1H, -NCH), 1.35 (t, 3H, $^{3}J_{HH}$ = 6.1 Hz, -CH_3); ^{13}CNMR (δ ppm): 168.6 (CO), 157.6 (C-8), 138.1 (C-6), 132.7 (C-11), 130.2 (C-7), 128.6 (C-9), 123.1 (C-10), 69.3 (C-1 & C-5), 52.6 (Ar-OCH_3), 53.2 (-OCH_3), 56.2 (C-5´), 133.9 (C-6´), 128.7 (C-7´ & 11´), 127.2 (C-8´ & 10´), 125.9 (C-9´), 54.4 (-OCH_2), 14.5 (-CH_3); ^{31}PNMR (δ ppm): 62.5 ppm; *Anal*. Calcd for $C_{19}H_{22}NO_5PS$: C, 56.01; H, 5.44; N, 3.44, Found C, 55.93; H, 5.39; N, 3.39.

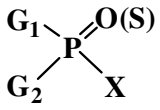
4.11. Methyl -3-(1H-3-indolyl)-2-[(7-methoxy-3-thioxo-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl)amino]propanoate (4j). Yield 72%; mp 190-192 °C. IR (KBr) (ν_{max} cm^{-1}): 3429 (N-H), 1738 (C=O), 791 (P=S); ^{1}HNMR (δ ppm): 8.60-8.75 (brs, 2H, NH), 7.10-7.60 (m, 8H, Ar-H), 5.79 (dd, 2H, $^{3}J_{HP}$ = 21.4 Hz and $^{2}J_{HH}$ = 14.2 Hz, 1,5-H), 4.91 (dd, 2H, $^{3}J_{HP}$ = 21.4 Hz and $^{2}J_{HH}$ = 14.2 Hz, 1,5-H), 3.61 (3H, s, -OCH_3), 3.81 (s, 3H, Ar-OCH_3), 3.38 (m, 1H, -NCH), 2.91 (d, 2H, $^{3}J_{HH}$ = 6.3 Hz, -CH_2-Ar); ^{31}PNMR (δ ppm): 60.9 ppm; *Anal*. Calcd for: *Anal*. Calcd for $C_{21}H_{23}N_2O_5PS$: C, 56.49; H, 5.19; N, 6.27, Found C, 56.40; H, 5.13; N, 6.21.

4.12. Ethyl 1-(7-methoxy-3-thioxo-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-yl)-1H-2-indolecarboxylate (4k). Yield 69%; mp 123-124 °C. IR (KBr) (ν_{max} cm^{-1}): 1729 (C=O), 787 (P=S); ^{1}HNMR (δ ppm): 7.09-7.61 (m, 8H, Ar-H), 5.78 (dd, 2H, $^{3}J_{HP}$ = 20.8 Hz and $^{2}J_{HH}$ = 13.6 Hz, 1,5-H), 4.88 (dd, 2H, $^{3}J_{HP}$ = 20.8 Hz and $^{2}J_{HH}$ = 13.6 Hz, 1,5-H), 4.23 (q, 2H, $^{3}J_{HH}$ = 6.1 Hz, -OCH_2), 3.80 (s, 3H, Ar-OCH_3), 1.31 (t, 3H, $^{3}J_{HH}$ = 6.1 Hz, -CH_3); ^{31}PNMR (δ ppm): 60.6 ppm; *Anal*. Calcd for: $C_{20}H_{20}NO_5PS$: C, 57.55; H, 4.83; N, 3.36. Found C, 57.47; H, 4.78; N, 3.60.

5. Bioactivity

5.1. Structure-Function Relationships of organophosphorus compounds (OPC):

Schrader-Clark[14] proposed that organophosphorus compounds containing the main pharmacophoric structure unit (**Figure-2**) may have significant biological activity. Slight variation in pharmacophoric structure (**Figure-2**) can have very drastic effects on the bioactive efficiency of organophosphorus compounds (OPC) due to the fact that an OPC substrate is very sensitive to the size, shape and polarity. These chemically and biologically variable parameters which are hard to estimate are involved in deciding "structure-activity" relationship of these compounds.



G_1 and G_2 = Groups which are difficult to displace from phosphorus

X = Fairly good leaving group

Figure 2. General pharmacophoric group of organophosphorus compounds

5.2. Antimicrobial Activity

Antimicrobial activity[15,16] of **4a-k** was tested against the growth of *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 55422) (Gram +ve) and *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (31488) (Gram –ve) using the disc diffusion method in nutrient agar medium which are spread by bacteria of 0.1 mL (10^5 CFU/mL) at two different concentrations (100 and 50 µg/mL) in dimethyl formamide (DMF). The plates of 8 mm were punched into the agar medium and filled with the title compound solutions to each filter paper disc and DMF was used as control. The plates were incubated at 35°C and examined for zone of inhibition around each disc after 24 h. The results were compared with the activity of the standard antibiotic Penicillin (50 µg/disc). All the compounds showed moderate to high bactericidal activity against both the bacteria (**Table 1**).

These compounds were also screened for antifungal activity against *Aspergillus niger* (ATCC 16404) and *Helminthosporium oryzae* (ATCC 11000) species along with the standard antifungal drug Griseofulvin (**Table 2**) utilizing the disc diffusion method at two different concentrations (100 and 50 µg/mL). Fungal cultures were grown on potato dextrose agar plates (PDA) were incubated for 72 h at 25 °C and spore suspension was adjusted to 10^5 spores/mL. Each test was done in triplicate and the mean of the diameter of the inhibition zones was calculated. Controls included the use of the solvent DMF without test compounds: no antibacterial activity was noticed for the solvent (DMF) employed in the test. The antimicrobial activity was evaluated by measuring the zone of inhibition against test organisms.

The minimum bactericidal concentration of tested compound 4a-k was 5 mm-16 mm of zone of inhibition with 50-100 µg/mL, respectively while minimum fungicidal concentration of tested compound 4a-k was 6 mm-11mm of zone of inhibition with 50-100 µg/mL, respectively.

Table 1. Antibacterial activity of compounds 4a-k (µg/mL)

Compound	Zone of Inhibition (mm)							
	K. pneumoniae		***B. subtilis***		***E. coli***		***S. aureus***	
	100 µg/mL	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**
4a	10	7	12	8	9	8	8	5
4b	12	9	13	9	9	8	8	7
4c	11	8	11	7	10	8	9	7
4d	12	9	12	8	11	9	10	9
4e	9	6	10	7	10	8	9	7
4f	11	9	11	8	10	7	9	7
4g	10	7	12	8	11	8	10	7
4h	11	9	11	6	10	7	8	5
4i	12	8	13	8	9	6	8	5
4j	15	10	13	10	10	8	9	6
4k	16	11	15	11	8	6	7	5
Penicillin[a]	15	12	16	13	12	8	10	7

[a]Reference Compound

It is gratifying to observe that majority of the compounds exhibited moderate to high antifungal activity when compared with that of Griseofulvin. Thus a new group of compounds with very high antibacterial and antifungal activity comparing well with presently commercial bactericides and antifungal drugs have been discovered.

Table 2. Antifungal activity of compounds 4a-k (µg/mL)

Compound	Zone of inhibition (mm)			
	Aspergillus niger		***Helminthosporium oryzae***	
	100 (µg/mL)	**50 (µg/mL)**	**100 (µg/mL)**	**50 (µg/mL)**
4a	8	6	8	7
4b	9	7	9	7
4c	9	8	8	7
4d	11	8	10	8
4e	10	8	9	7
4f	10	6	10	8
4g	11	8	11	8
4h	10	7	10	8
4i	9	6	9	7
4j	11	7	10	8
4k	8	6	9	6
Griseofulvin[a]	12	7	12	9

[a] Reference Compound

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