Synthesis and antimicrobial activity of novel (3a,*S*)-1-(aminoacid ester)-3a,4-dihydro-3*H*-1λ^5-[1,3,2] oxazaphospholo[3,4-*a*] indol-1-oxides

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Abstract

Synthesis of (3a,*S*)-1-(amino acid ester)-3a,4-dihydro-3*H*-1λ^5-[1,3,2]oxazaphospholo[3, 4-*a*]indol-1-oxides was accomplished through a two-step process involving preparation of the monochloride and its subsequent reaction with amino acid ester hydrochlorides in dry tetrahydrofuran in the presence of triethylamine at various temperatures. All the title compounds were characterized by IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data. They exhibited significant antimicrobial activity.

Keywords: (2S)-2,3-Dihydro-1*H*-2-indolyl methanol, amino acid esters, triethylamine, (3a,*S*)-1-chloro-3a, 4-dihydro-3*H*-1λ^5-[1,3,2] oxaazaphospholo[3,4-*a*]indol-1-oxides, antimicrobial activity

Introduction

Phosphoramides substituted with an amino acid ester are an important class of rationally designed therapeutics for antineoplastic properties.[1-5] The attachment of an amino acid group to the phosphate moiety is expected to increase cellular uptake and thus enhance chemotherapeutic properties. Organophosphorus compounds are used as insecticides, agricultural and horticultural pesticides and veterinary medicines. They are also used in human medicines and in various public hygiene products for use both by professional operators and the general public. Pesticides in soil have far reaching consequences as they disturb the delicate equilibrium between microorganisms and their environment.[6] Bioremediation is a promising area which holds potential for eco-restoration of pesticide contaminated soil. Several microorganisms are able to degrade a large variety of compounds.[7]

The presence of an exocyclic P-N bond in an amino acid ester attached to a benzoxazaphosphorin system is expected to increase cellular uptake of their chemotherapeutic

properties. Further, hydrolysis of these novel heterocycles may release products of limited toxicity to the system. In view of this, we have synthesized new heterocycles in which amino acid esters are linked to a phosphorus atom, studied their antimicrobial activity and phosphate degradation potential in several bacterial species.

Results and Discussion

Synthesis of the compounds (**5a-h)** was accomplished through two steps. Reaction of (2*S*)-2,3-dihydro-1*H*-indol-2-ylmethanol (**1**) with phosphorus oxychloride in equimolar quantities in the presence of triethylamine in dry tetrahydrofuran at 10-30 °C produced the corresponding monochoride, (3a,*S*)-1-chloro-3a,4-dihydro-3*H*-1λ^5-[1,3,2]oxaza- phospholo[3,4-*a*]indol-1-oxide (**3**). This was subsequently reacted with various amino acid ester hydrochlorides, in dry tetrahydrofuran in the presence of triethylamine to obtain the title compounds in moderate yields (52-69%).

The second step of the reaction was run at 20-40 °C with stirring for 6-8 h, with progress monitored by TLC. In all the reactions, the crude title compounds were separated by removing the solvent from the filtrate in a rota-evaporator. They were purified by flash chromatography using hexane-ethyl acetate (6**:**2) step gradient mixtures as eluents and characterized by elemental analysis, IR, ^{1}H, ^{13}C and ^{31}P NMR and mass spectral data.[8]

Compounds **5a-h** showed absorption bands in the region 1215-1282 cm^{-1} for (P=O) and 3446-3408 cm^{-1} for (P-NH) confirming the presence of phosphoryl (P=O) and P-NH functional groups in these compound.[12] The aromatic protons of **5a-h** resonated as multiplets at δ 7.67-6.94. The C-4 methylene protons resonated as multiplets at δ 4.46-4.02 indicating their non-equivalence and coupling with phosphorus[12,13] The ^{13}C NMR chemical shifts of **5a, 5b, 5e** and **5h** were interpreted on the basis of additivity rules. The phosphorus bonded C-3 resonated as a singlet at δ 52.7-59.7.The chiral carbon (C-3a) gave a singlet in the region δ 45.4-45.6. The remaining carbon signals were observed in the expected region.[8] The ^{31}P NMR spectra of **5a-h** showed phosphorus resonance signals[8,9] in the region 2.16 to 12.12 ppm.[10] Thus, the combined analytical, IR and NMR data agreed conclusively with the proposed structures for **5a-h**.

Antimicrobial activity

The compounds **5a-h** were screened for their antibacterial activity against *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram -ve) by the disc diffusion method[12,13] in nutrient agar medium at three different concentrations (25, 50, 100 g/disc) in dimethyl formamide (DMF). These solutions were added 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 Streptomycin (50 μg/disc).Their antifungal activity was evaluated against *Aspergillus niger* and *Helminthosporium oryzae* at three different concentrations (100, 50, 25 μg /disc) and Bavestin was used as the reference compound. Fungal

cultures were grown on potato dextrose broth at 25 °C and spore suspension was adjusted to 10^5 spores/mL. Most of the compounds showed moderate activity against bacteria and low activity on fungi. 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.

Conclusions

In summary, we have reported an effective and simple reaction for the synthesis of novel phosphorus heterocyclic compounds containing a chiral centre with amino acid moieties linked to phosphorus. The majority of the compounds (**5a-h**) exhibited moderate activity against bacteria and fungi when compared to that of the respective standards.

Experimental Section

General procedures. All melting points were determined in open capillary tubes on a Mel-temp apparatus and are uncorrected. Microanalysis was performed at the Central Drug Research Institute, Lucknow, India. Infrared spectra (ν_{max} in cm^{-1}) were recorded as KBr pellets on a Perkin-Elmer 283 double beam spectrophotometer. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on AMX 400 MHz spectrometer operating at 400 MHz for ^1H, 100 MHz for ^{13}C, and 161.9 MHz for ^{31}P, using deuterochloroform as solvent. The ^1H and ^{13}C NMR chemical shifts were referenced to tetramethylsilane, and ^{31}P chemical shifts to 85% H_3PO_4. Mass spectra were recorded on a Jeol SX 102 DA/600 mass spectrometer using Argon/Xenon (6 KV, 10 mA) as the FAB gas and also on a Shimadzu QP-2000 GC-MS instrument. Refractive indices were recorded using a SEPA-300 instrument and a 10 cm tube at 28 °C.

(2*S*)-2, 3-Dihydro-1*H*-2-indolyl methanol (1)

To a stirred and ice-cold suspension of $LiAlH_4$ (3.10 g, 0.0817 mol) in dry THF (100 mL), a solution of ethyl *2S*-indole-2-carboxylate (7.60 g, 0.039 mole) in dry THF (35 mL) was added dropwise. The mixture was stirred and refluxed for 3 h and then cooled, and the excess of $LiAlH_4$ was decomposed by the addition of a mixture of water (6.2 mL) and THF (50 mL). After stirring at room temperature for 1 h, the inorganic salts were filtered off and washed with hot ethyl acetate (150 mL). The combined organic filtrate and washings were dried (Na_2SO_4) and evaporated under reduced pressure to give the crude amino alcohol[14] as a crystalline product, which was filtered off, washed with Et_2O and recrystallised from EtOAc, yield: 4.03 g (68%), m.p. 127 °C.

Proline ethyl ester hydrochloride (3a)

Thionyl chloride (0.7 mL, 0.01 mol) was added slowly to methanol (25 mL) at 0 °C then proline (1.31 g, 0.01 mol) was dissolved in it. The solution was refluxed for 4 hours. Solvent was removed in a rota-evaporator, the crude proline methyl ester hydrochloride, was triturated with ether at 0 °C until excess dimethyl sulfite was removed. The resulting solid product was collected and dried under vacuum, to give crude methyl ester hydrochloride. The crude material was dissolved in minimum amount of hot methanol. Slow addition of excess of ether followed by cooling to 0 °C gave pure crystals. They were washed twice with ether:methanol (5:1) and dried under vacuum to get proline methyl ester hydrochloride,[15] yield 25 g (80%), mp 148-150 °C.
Other amino acid esters were prepared using the above procedure.

(3a,*S*)-1-Chloro-3a,4-dihydro-3*H*-1λ^5-[1, 3, 2]oxazaphospholo[3, 4-*a*]indol-1-oxide (3)

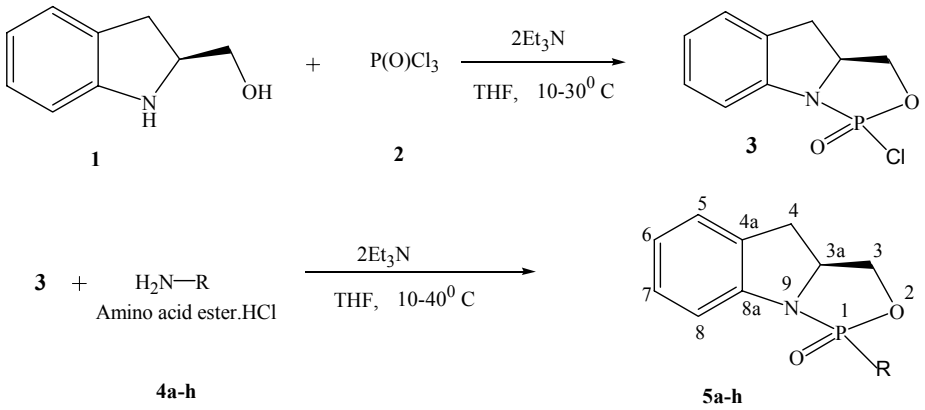
A solution of phosphorus oxychloride (0.02 mol) in 10 mL of dry tetrahydrofuran was added dropwise to a stirred solution of (**1)** (0.02 mol) and triethylamine (0.04 mol) in 20 mL of dry tetrahydrofuran at 0 °C over a period of 30 min. After stirring for 6 h at 10-30 °C, formation of the intermediate, (3a,*S*)-1-chloro-3a,4-dihydro-3*H*-1λ^5-[1,3,2]oxazaphospholo[3,4-*a*]indol-1-oxide (**3**) was ascertained by TLC analysis. Triethylamine hydrochloride was separated by filtration and the reaction mixture was used for the next reaction step without further purification.

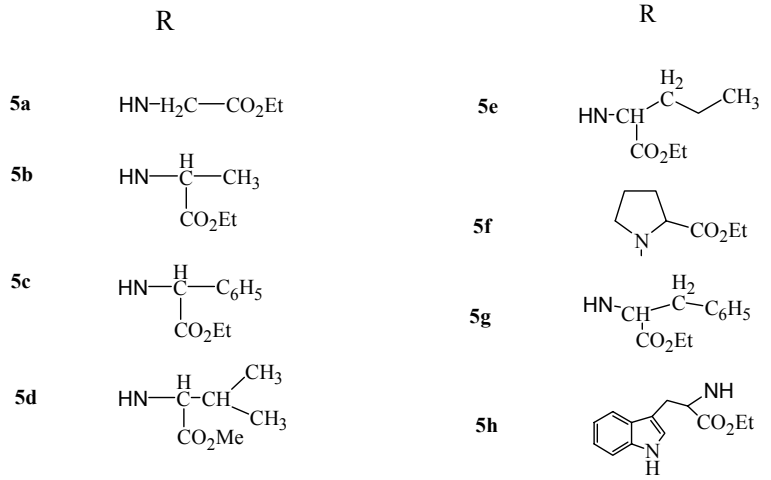
Typical Procedure. (3a,*S*)-1-(Glycine ethyl ester)-3a,4-dihydro-3*H*-1λ^5-[1, 3, 2] oxazaphospholo[3,4-*a*]indol-1-oxide (5a)

Glycine ethyl ester (0.02 mol) in 10 mL of tetrahydrofuran was added to the reaction mixture of **3** at 10 °C with stirring in the presence of triethylamine (0.04 mol). The reaction mixture was slowly raised to 40 °C and maintained at this temperature for 5 h with stirring. The progress of the reaction was monitored by TLC. After the completion of the reaction, triethylamine hydrochloride was separated by filtration. The solvent was evaporated under reduced pressure to obtain a solid residue, which was washed with water and recrystallised from methanol to yield the title compound (**5a**). Compounds **5b-h** were prepared by the above procedure.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) for the compounds **5a-h.** Compounds **5a-h** concentrations of 0.1-5.6 μg/mL in steps of 100 μg/mL were evaluated. Specifically 0.1 mL of standardized inoculum (1.2 x 10^7 CFU/mL) was added to each tube. The tubes were incubated aerobically at 37 °C for 18-24 h. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing compounds **5a-h** and the growth medium without inoculum) and organism control (the tube containing and growth medium, physiological saline and the inoculum). The lowest concentration (highest dilution) of the compounds **5a-h** that produced no visible bacterial growth (no turbidity) when compared with the control tubes was regarded as MIC [16] (Table 2).





Scheme 1

Physical, analytical and spectral data for the 5a-h

(3a,*S*)-1-(Glycine ethyl ester)-3a,4-dihydro-3*H*-1λ^5-[1,3,2]oxazaphospholo[3,4-*a*] indol-1-oxide (5a). Yield 60%, m.p. 120-122 °C. IR (KBr): ν_{max} 3412 (NH), 1258 (P=O), 1746 (C=O) cm^{-1}; ^{31}P NMR ($CDCl_3$, 161.9 MHz) δ: 6.06, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.02-7.48 (m, 4H, Ar-H), 3.08 (d, *J* = 6.5 Hz, 2H, CH_2), 4.16-4.27 (m, 1H, CH), 9.84 (br s , 1H, P-N-H), 3.78 (d, *J* = 31.4 Hz, 2H, -OCH_2-), 3.65 (q, 2H, O$\underline{CH_2}CH_3$), 2.36 (s,2H,CH_2), 1.21 (t, 3H, $OCH_2C\underline{H_3}$), ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 58.3 (C-3), 45.6 (C-3a), 39.4 (C-4), 129.8 (C-4a), 122.4 (C-5), 124.6 (C-6), 113.7 (C-7), 128.9 (C-8), 138.4 (C-8a), 174.5 (C=O), 53.4 (CH_2), 49.8 (OCH_2), 17.9 (OCH_2CH_3); Anal. Calcd. for $C_{13}H_{17}N_2O_4P$: C, 52.70; H, 5.78; N, 9.46. Found: C, 52.65; H, 5.74; N, 9.42%, $[\alpha]_D$ (+) 3.0 .

(3a,*S*)-1-(Alanine ethyl ester)-3a,4-dihydro-3*H*-1λ^5-[1,3,2]oxazaphospholo[3,4-*a*] indol-1-oxide (5b). Yield 65%, m.p. 135-137 °C. IR (KBr): ν_{max} 3418 (NH), 1246 (P=O), 1754 (C=O) cm^{-1}; ^{31}P NMR ($CDCl_3$, 161.9 MHz) δ: 12.02, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.09-7.58 (m, 4H, Ar-H), 3.03 (d, *J* = 6.2 Hz, 2H, CH_2), 4.06-4.14 (m, 1H, CH), 9.88 (br s , 1H, P-N-H), 3.72 (d, *J*

= 31.4 Hz, 2H, -OCH_2-), 3.48 (q, 2H, OCH_2CH_3), 1.19 (t, 3H, OCH_2CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 59.4 (C-3), 45.4 (C-3a), 39.8 (C-4), 129.6 (C-4a), 122.9 (C-5), 124.4 (C-6), 113.8 (C-7), 129.7 (C-8), 136.4 (C-8a), 170.8 (C=O), 53.6 (CH), 48.7 (OCH_2), 17.4 (OCH_2CH_3); Anal. Calcd. for $C_{14}H_{19}N_2O_4P$: C, 54.19; H, 6.17; N, 9.03% Found: C, 54.13; H, 6.14; N, 9.00%, $[\alpha]_D$ (+) 3.4.

(3a,*S*)-1-(Phenyl glycine ethyl ester)-3a,4-dihydro-3*H*-1λ^5-[1,3,2] oxazaphospholo- [3,4-*a*]indol-1-oxide (5c). Yield 52%, m.p. 147-149 °C. IR (KBr): ν_{max} 3446 (NH), 1242 (P=O), 1744 (C=O) cm^{-1}; ^{31}P NMR ($CDCl_3$, 161.9 MHz) δ: 2.16, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.09-7.49 (m, 9H, Ar-H), 3.03 (d, *J* = 6.4 Hz, 2H, CH_2), 4.02-4.09 (m, 1H, CH), 9.81 (br s , 1H, P-N-H), 3.87 (d, *J* = 32.1 Hz, 2H, -OCH_2-), 4.48-4.56 (m, 1H, CH), 3.65 (q, 2H, OCH_2CH_3), 1.13 (t, 3H, OCH_2CH_3), 1.19 (d, 2H, -CH_2); Anal.Calcd. for $C_{19}H_{21}N_2O_4P$: C, 61.29; H, 5.68; N, 7.52% Found: C, 61.25; H, 5.64; N, 7.50%, $[\alpha]_D$ (+) 2.5 .

(3a,*S*)-1-(Valine methyl ester)-3a,4-dihydro-3*H*-1λ^5-[1,3,2]oxazaphospholo[3,4-*a*] indol-1-oxide (5d). Yield 68%, m.p. 172-175 °C. IR (KBr): ν_{max} 3435 (NH), 1282 (P=O), 1752 (C=O) cm^{-1}; ^{31}P NMR ($CDCl_3$, 161.9 MHz) δ: 6.62, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.08-7.67 (m, 4H, Ar-H), 3.22 (d, *J* = 6.4 Hz, 2H, -CH_2-), 4.28-4.46 (m, 1H, -CH), 9.78 (s, 1H, P-NH), 3.75 (d, *J* = 33.8 Hz, 2H, -OCH_2-), 4.62-4.78 (m, 1H, -CH-), 3.54 (q, 2H, -OCH_2-), 1.17 (t, 3H, -CH_3); Anal.Calcd. for $C_{15}H_{21}N_2O_4P$: C, 55.55; H, 6.53; N, 8.64% Found: C, 55.50; H, 6.48; N, 8.60 %, $[\alpha]_D$ (+) 2.9.

(3a,*S*)-1-(nor-Valine ethyl ester)-3a,4-dihydro-3*H*-1λ^5-[1,3,2] oxazaphospholo[3,4-*a*] indol-1-oxide (5e). Yield 67%, m.p. 130-132 °C. IR (KBr): ν_{max} 3442 (NH), 1261 (P=O), 1743 (C=O) cm^{-1}; ^{31}P NMR ($CDCl_3$, 161.9 MHz) δ: 7.96, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.09-7.67 (m, 4H, Ar-H), 3.04 (d, *J* = 6.2 Hz, 2H, CH_2), 4.18-4.26 (m, 1H, CH), 9.02 (br s , 1H, P-N-H), 3.91 (d, *J* = 31.4 Hz, 2H, -OCH_2-), 4.58-4.74 (m, 1H, CH), 3.46 (q, 2H, OCH_2CH_3), 1.39 (t, 3H, OCH_2CH_3), 1.51-1.58 (m, 2H, CH_2), 1.72-1.79 (m, 2H, CH_2), 1.09 (t, CH_3), ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 59.7 (C-3), 45.4 (C-3a), 39.7 (C-4), 131.7 (C-4a), 122.0 (C-5), 124.3 (C-6), 114.1 (C-7), 129.8 (C-8), 142.8 (C-8a), 172.3 (C=O), 51.7 (OCH_2), 54.4 (NH-CH), 17.6 (OCH_2CH_3), 13.3 (CH_2), 13.8 (CH_2), 18.0 ($CH_2CH_2CH_3$); Anal.Calcd. for $C_{16}H_{23}N_2O_4P$: C, 56.80; H, 6.85; N, 8.28% Found: C, 56.75; H, 6.80; N, 8.20%, $[\alpha]_D$ (+) 3.1.

(3a,*S*)-1-(Proline ethyl ester)-3a,4-dihydro-3*H*-1λ^5-[1,3,2]oxazaphospholo[3,4-*a*] indol-1-oxide (5f). Yield 65%, m.p. 160-163 °C. IR (KBr): ν_{max} 3408 (NH), 1215 (P=O), 1746 (C=O) cm^{-1}; ^{31}P NMR ($CDCl_3$, 161.9 MHz) δ: 5.82, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.08-7.42 (m, 4H, Ar-H), 3.19 (d, *J* = 6.4 Hz, 2H, CH_2), 4.13-4.24 (m, 1H, CH), 9.58 (br s , 1H, P-N-H), 3.74 (d, *J* = 32.4 Hz, 2H, -OCH_2-), 3.36 (q, 2H, OCH_2CH_3), 1.17 (t, 3H, OCH_2CH_3), 1.91-2.02 (m, 2H, CH_2), 1.64-1.79 (m, 2H, CH_2), 2.02-2.21 (t, 2H, CH_2); Anal.Calcd. for $C_{16}H_{22}N_3O_4P$: C, 54.70; H, 6.31; N, 11.96. Found: C, 54.68; H, 6.29; N, 11.92%, $[\alpha]_D$ (+) 3.5.

(3a, S)-1-(Phenyl alanine ethyl ester)-3a,4-dihydro-3H-1λ^5-[1,3,2] oxazaphospholo- [3,4-a] indol-1-oxide (5g). Yield 56%, m.p. 160-162 °C. IR (KBr): ν_{max} 3442 (NH), 1272 (P=O), 1736 (C=O) cm^{-1}, ^{31}P NMR ($CDCl_3$, 161.9 MHz) δ: 5.02, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.10-7.67 (m, 9H, Ar-H), 3.03 (d, *J* = 6.4 Hz, 2H, CH_2), 4.19-4.29 (m, 1H, CH), 9.86 (br s , 1H, P-N-H),

3.65 (d, *J* = 32.2 Hz, 2H, -OCH_2-), 4.54-4.68 (m, 1H, CH), 3.83 (q, 2H, OCH_2CH_3), 1.15 (t, 3H, OCH_2CH_3), 1.19 (d, 2H, CH_2); Anal. Calcd. for $C_{20}H_{23}N_2O_4P$: C, 62.17; H, 6.00; N, 7.25% Found: C, 62.13; H, 5.95; N, 7.20%, $[\alpha]_D$ (+) 2.7.

(3a,*S*)-1-(Tryptophan ethyl ester)-3a,4-dihydro-3*H*-1λ^5-[1,3,2]oxazaphospholo[3,4-*a*]indol-1-oxide (5h). Yield 69%, m.p. 146-148 °C. IR (KBr): ν_{max} 3421 (NH), 1226 (P=O), 1742 (C=O) cm^{-1}; ^{31}P NMR ($CDCl_3$, 161.9 MHz) δ: 5.96, ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.94-7.67 (m, 8H, Ar-H), 3.05 (d, *J* = 6.3 Hz, 2H, CH_2), 4.12-4.21 (m, 1H, CH), 9.67 (br s , 1H, P-N-H), 10.17 (br s, 1H, Ar-N-H), 3.65 (d, *J* = 31.9 Hz, 2H, -OCH_2-), 3.65 (q, 2H, OCH_2CH_3), 1.17 (t, 3H, OCH_2CH_3), 3.72-3.86 (m, 1H, CH-CH_2), 3.91 -4.08 (m, 1H, CH-CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 52.7 (C-3), 45.5 (C-3a), 39.7 (C-4), 130.4 (C-4a), 122.1 (C-5), 124.4 (C-6), 113.9 (C-7), 129.6 (C-8), 136.1 (C-8a), 169.7 (C=O), 52.5 (OCH_2), 21.1 (OCH_2CH_3), 56.1 (CH-N), 39.9 (NH-CH_2), 39.7 (NH-CH_2-CH), 39.2 (NH-CH-CH_2), 124.6 (C-1′), 126.4 (C-2′), 131.6 (C-3′), 111.5 (C-4′), 136.1 (C-5′), 148.8 (C-6′); Anal.Calcd. for $C_{22}H_{27}N_4O_4P$: C, 59.72; H, 6.15; N, 12.66% Found: C, 59.65; H, 6.10; N, 12.60%, $[\alpha]_D$ (+) 2.2 .

Table 1. Antimicrobial activity of compounds (5a-h) in terms of DIZ in mm

Compd	Zone of inhibition (mm)															
	Aspergillus niger μg/mL				*Helminthosporium oryzae* μg/mL				*Escherichia coli* μg/mL				*Staphlococcus aureus* *μg/mL*			
	100	50	SD	25	100	50	SD	25	100	50	SD	25	100	50	SD	25
5a	8	5	1.414	2	11	5	1.414	2	10	6	2.081	2	9	7	2.828	3
5b	11	8	3.535	3	14	9	4.760	4	7	8	3.535	1	7	6	2.081	1
5c	10	6	2.081	3	12	6	2.081	3	13	4	2.738	-	12	4	2.738	2
5d	12	5	1.414	1	10	5	1.414	-	9	1	1.000	2	5	8	3.535	-
5e	10	7	2.828	1	-	-	1.414	-	7	3	2.581	-	6	5	1.414	2
5f	11	7	2.828	2	11	5	2.828	1	10	5	1.414	3	13	5	1.414	1
5g	10	5	1.414	1	13	7	3.535	2	9	7	2.828	2	8	6	2.081	-
5h	9	6	2.081	3	14	8	2.828	1	12	4	2.738	1	10	7	2.828	1
Bavestin	15	9	4.760	5	16	10	6.300	5								
Strepto-mycin									15	9	4.760	5	16	10	6.300	5

"-" indicates no activity.

SD Standard deviation.

Table 2. Minimum inhibitory concentration for 5a-h (MIC), μg/mL

Compd.	*Aspergillus niger*	*Helminthosporium oryzae*	*Escherichia coli*	*Staphylococcus aureus*
5a	3.6	4.0	3.3	3.5
5b	4.0	3.9	4.2	3.8
5c	3.9	5.3	4.1	4.0
5d	4.5	4.6	4.5	5.3
5e	3.2	3.8	3.8	3.0
5f	4.7	4.2	4.0	4.5
5g	3.5	3.5	2.7	3.9
5h	4.6	3.2	3.5	4.2

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