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Synthesis and pharmacological evaluation of novel (3aS)-2-substituted perhydro-2λ^5-pyrrolo[1,2-c][1,3,2]oxazaphosphole-2-thiones as antioxidants and antimicrobial agents

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ABSTRACT

The present communication reveals the synthesis of novel (3aS)-2-substituted perhydro-2λ^5-pyrrolo[1,2-c][1,3,2]oxazaphosphole-2-thiones, **(6a-n)**. The synthetic pathway comprises two steps i.e., the synthesis of various (thio)phosphorodichlorides **(5a-n)** from thiophosphoryl chloride **(3)** and amino compounds **(4a-l)** and two drugs zidovudine **(4m)** and lamivudine **(4n)**. They were further cyclized with (S)-(+)-prolinol **(2)** to yield the title compounds **(6a-n)**. Here (S)-(+)-prolinol **(2)** is synthesized by the asymmetric reduction of S)-(+)-proline **(1)** with lithium borohydride in the presence of trimethylsilyl chloride. All the newly synthesized compounds were characterized by spectral data, microanalyses and screened for their antioxidant and antimicrobial activity. A few of them were found to possess significant antioxidant and antimicrobial activities.

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KEYWORDS

L-prolinol;
Thiophosphoryldichlorides;
1,3,2-oxazaphosphole-2-thione derivatives;
Antioxidant activity;
Antimicrobial activiy.

INTRODUCTION

The inceasing interest in the field of organophosphorus heterocyclic chemistry is due to their unique physico chemical properties[1] and potential biological activities[2,3]. Various classes of phosphorus heterocycles containing P-O, P-N moieties such as cyclophosphamide and its derivatives are also antitumor agents[4]. In view of the potential bioactivity of these moieties, their phosphorus structural analogues[5], which are a class of heterocycles have been synthesized. The synthesis of multi-ring phosphorus heterocycles were attracted much importance as they find applications in medicine and industry[6]. This class of compounds are bioactive and the proline based oxazaborolidines had been used as catalysts by Corey for the borane-mediated enantioselective reduction of prochiral ketones, a plethora of oxazaborolidines and related catalysts based on various chiral pool sources have been developed and their applications have been well studied[7-10]. Simillarly proline-based phosphorylated derivatives were used for borane mediated reduction of prochiral α-halo ketones to α-halo alcohols[11]. It is noted that phosphorus compounds with N-P=O

structural frame work containing different bulky amino and phenolic groups on phosphorus as substituents were used in borane-mediated asymmetric reduction of prochiral ketones with high enantiomeric purity, where the basic cyclic moiety controls the stereochemical course of the reaction, while the groups on the phosphorus have little significant role in directing the stereochemical course of the reaction[11]. These aspects are driven to design and synthesis of title compounds as phosphorylated derivatives of (S)-(+)-prolinol as a part of our research and evaluated their antioxidant and antimicrobial activity.

EXPERIMENTAL

Chemistry

The chemicals were procured from Sigma-Aldrich, Merck and Lancaster and used as such without further purification. All solvents used for the spectroscopic and other physical studies were reagent grade and further purified by literature methods[12]. Melting points (m.p.) were determined in open capillary tubes using a calibrated thermometer by Guna Digital Melting Point apparatus, expressed in degrees centigrade (°C) and are uncorrected. Specific rotations (in degrees,°) were recorded in methanol on a Perkin-Elmer Model 241 polarimeter at the sodium D line. Infrared spectra (IR) were obtained on a Perkin-Elmer Model 281-B spectrophotometer. Samples were analyzed as potassium bromide (KBr) disks. Absorptions were reported in wave numbers (cm^{-1}). ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded as solutions in DMSO-d_6 on a Bruker AMX 500 MHz spectrometer operating at 500 MHz for ^{1}H, 125 MHz for ^{13}C and 202 MHz for ^{31}P NMR. The ^{1}H and ^{13}C chemical shifts were expressed in parts per million (ppm) with reference to tetramethylsilane (TMS) and ^{31}P chemical shifts to 85 % H_3PO_4. LCMS mass spectra were recorded on a Jeol SX 102 DA/ 600 Mass spectrometer. Elemental analysis was performed on Thermo Finnigan Insturment at University of Hyderabad, Hyderabad.

Pharmacology

Antioxidant activity was performed with two methods DPPH scavenging and Super Oxide Dismutase scavenging activities. Scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm.

The compounds were assayed for antimicrobial activity against bacterial and fungal cultures. The bacteria includes Gram positive (*Staphyloccus aureus, Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae*) and fungal cultures (*Aspergillus niger* and *Candida albicans*). The bacterial cultures were grown in nutrient agar media and sub cultured and fungal cultures have been grown on the Potato Dextrose Agar media for the better growth and sub cultured on to the petri plates for the experiments. The bacterial and fungal culture containing discs were placed on the media. The Petri plates with bacterial cultures were incubated at 37°C for 24 hrs and fungal cultures were incubated for 3-4 days for better observation. The zone of inhibition was measured where the plaques were formed. All the experiments were carried out in triplicates and the results were expressed as MIC values in mm.

RESULTS AND DISCUSSION

Chemistry

Differently substituted novel (3aS)-2-substituted perhydro-2λ^5-pyrrolo[1,2-c] [1,3,2] oxazaphosphole-2-thiones were obtained by the two step reaction, comprising the preparation of the substituted (thio) phosphorodichlorides by the reaction of various amines with thiophosphoryl chloride at 5-10°C in THF and TEA for 1 hour, next they were used for cyclization of the (S)-(+)-Prolinol at 30- 40°C in the presence of TEA to yield the title compounds. The resulted reaction mixture was filtered to remove the triethylammonium chloride. The solvent was removed in a rota-evaporator to get the crude products. They were further purified by column chromatography. The title compounds were obtained with moderate to high yields (60-80%) (Scheme 1).

Synthesis of L-prolinol

The pure optically active L-proline is converted to L-prolinol by the following the reported procedure.[13] A solution of Me_3SiCl (8.64 g, 80 mmol) was added under Nitrogen atmosphere to a solution of $LiBH_4$ (0.87 g, 40 mmol) in THF (20 mL) over the course of 5 min. A

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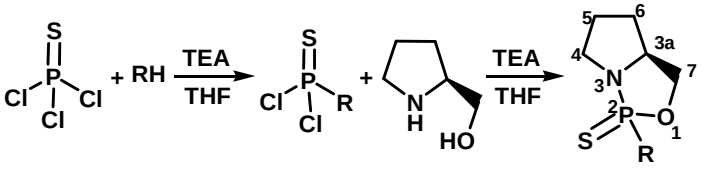

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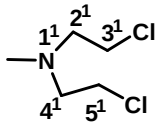
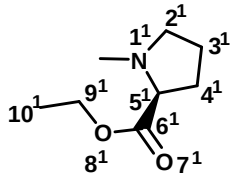
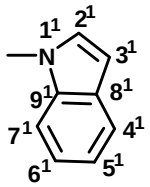
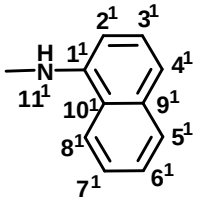
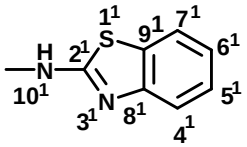
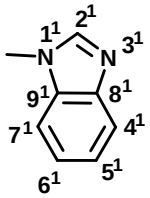
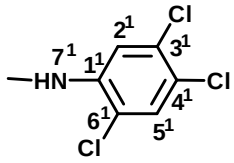
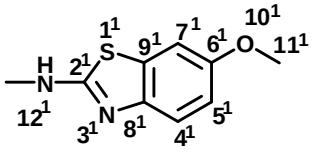
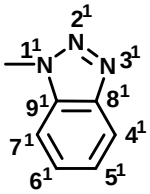
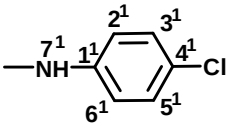
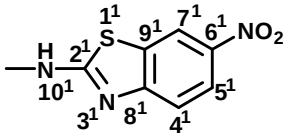
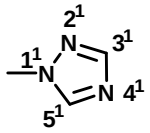
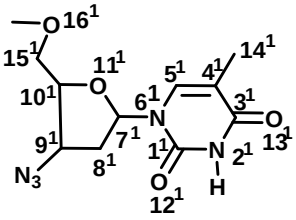
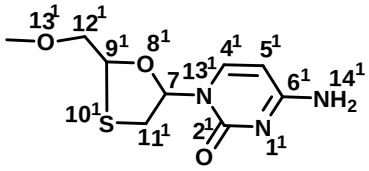
6a		6e		6i	
6b		6f		6j	
6c		6g		6k	
6d		6h		6l	
6m		6n			

Scheme 1

precipitate of LiCl and a borane-THF complex was formed. This borane-THF complex with the assistance of excess Me_3SiCl, acts as the reducing agent to reduce the L-proline. To the same reaction mixture, L-proline (2.34 g, 20 mmol) was added portion wise to the mixture within 5 min. After stirring for 24 h at room temperature, 30 mL of MeOH was cautiously added and the volatiles were removed by distillation. The residue was treated with 20% KOH solution and extracted three times with 50 mL portions of CH_2C1_2. The organic phases were combined, dried over Na_2SO_4, and the solvent was evaporated to afford L-Prolinol (Scheme 2) which was spectroscopically pure (^{1}H NMR). Yield after distillation was 1.87 g (86 %), $[\alpha]_D^{25}$ **=+14.7 (neat),** Here, the addition of Me_3SiC1 makes possible the reduction of optically pure α-amino acids with $LiBH_4$ in THF to optically pure β-amino alcohols because of its good solubility in THF and the milder reaction conditions.

The chemical structures of all the title compounds **(6a-n)** were characterized by IR, ^{1}H, ^{13}C, ^{31}P NMR, mass spectral data and elemental analyses and their data are presented in experimental section. Characteristic

IR stretching absorptions were observed in the regions 3304-3334 (N-H), 1688-1724 (C=O), 1658-1679 (C=N), 1323-1325 (O=C-O) 1092-1105 (P-O-$C_{Substituted}$), 1012-1041 (P-O-C_{Cyclic}), 1010-1035 (P-N-$C_{Substituted}$), 943-973 (P-N-C_{Cyclic}) and 763-791 cm^{-1} (P=S).[14] In ^{1}H NMR, the 3a proton resonated in the region 2.99-3.20 as a multiplet.[15] In the ^{13}C NMR, C_{3a} has resonated as a singlet in the region δ 45.1-48.1. The ^{31}P NMR chemical shifts were observed in the range of 19.23 to 21.42[16,17].

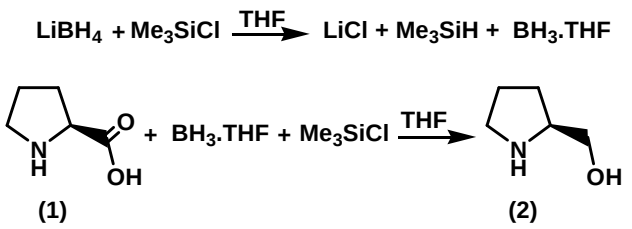
Synthesis of (3aS)-1-[(5-methoxy-1,3-benzithiazol-2-yl)amino] perhydro-1λ^5- pyrrolo [1,2-c] [1,3,2] oxazaphosphole-2-thione (6g)

5-Methoxybenzo[*d*]thiazol-2-yl phosphoramidothioic dichloride **(5g)** was prepared by the reaction of 5-methoxybenzo[d]thiazol-2-amine **(4g)** with thiophosphoryl chloride **(3)** (1.0 g, 5.95 mmol) at 0-5°C in THF and TEA (0.60 g, 5.95 mmol) for 1 hour. In the next step, **(5g)** was treated with L-prolinol **(2)** (0.60 g, 5.95 mmol) and TEA (1.20 g, 11.90 mmol) at 20°C and stirred at 35°C for 8 hours to yield (3aS)-1-[(5-methoxy-1,3-benzothiazol-2-yl)amino]perhydro-1λ^5-pyrrolo [1,2-c] [1,3,2] oxazaphosphole -2- thione. The reaction mixture was filtered to remove the triethylammonium chloride. The solvent from the filtrate was removed in a rota-evaporator and the crude product was collected and it was purified by column chromatography. The final product is obtained with 80% yield, m.p.274-276°C.

Spectral data of the synthesized compounds

(3a*S*)-1-[di(2-chloroethyl)amino)perhydro-2λ^5-pyrrolo[1,2-*c*][1,3,2]oxazaphosphole-2-thione (6a)

Pale yellow crystals. Yield: 79%. Mol.Wt.: 303. m.p.: 121-123°C. IR (KBr) ν cm^{-1}: 1017 (P-N-$C_{substituted}$), 1012 (P-O-C_{cyclic}), 943 (P-N-C_{cyclic}), 769 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.17 (t, 4H, H-3^l & H-5^l), 3.34 (t, 4H, H-2^l & H-4^l), 3.23-3.32 (m, 2H, H-7), 2.99-3.06 (m, 1H, H-3a), 2.81-2.89 (m, 2H, H-4), 1.45-1.65 (m, 4H, H-5 & H-6). ^{13}C-NMR (DMSO-d_6) δ ppm: 58.7 (C-7), 49.9 (C-2^l & C-4^l), 47.8 (C-3a), 44.8 (C-4), 43.1 (C-3^l & C-5^l), 33.5 (C-6), 27.8 (C-5). ^{31}P-NMR (DMSO-d_6) δ ppm: 21.20. LCMS (m/z): 307 [M+4], 305 [M+2], 303[M+·], 268, 240, 134. Anal. Calcd. for $C_9H_{17}Cl_2N_2OPS$: C, 35.65; H, 5.65; N, 9.24. Found: C, 35.48; H, 5.56; N, 9.08%.



Scheme 2

(3aS)-2-(1-naphthylamino)perhydro-2λ^5-pyrrolo [1,2-c][1,3,2]oxazaphosphole-2-thione (6b)

Brownish red crystals. Yield: 78%. Mol.Wt.: 304. m.p.: 150-153°C. IR (KBr) ν cm^{-1}: 3321 (N-H), 1022 (P-O-Ccyclic), 1018 (P-N-$C_{substituted}$), 952 (P-N-C_{cyclic}), 783 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.94-8.02 (m, 1H, H-8^l), 7.54-7.66 (m, 1H, H-5^l), 7.41-7.52 (m, 2H, H-6^l & C-7^l), 7.11-7.19 (m, 2H, H-3^l & H-4^l), 7.16 (s, 1H, H-11^l), 6.52-6.61 (m, 1H, H-2^l), 3.23-3.31 (m, 2H, H-7), 3.01-3.14 (m, 1H, H-3a), 2.80-2.89 (m, 2H, H-4), 1.42-1.61 (m, 4H, H-5 & H-6). ^{13}C-NMR (DMSO-d_6) δ ppm: 145.8 (C-1^l), 134.2 (C-10^l), 128.5 (C-5^l), 126.6 (C-3^l), 126.2 (C-6^l), 124.8 (C-7^l), 124.3 (C-9^l), 121.1 (C-8^l), 119.2 (C-4^l), 109.3 (C-2^l), 58.3 (C-7), 47.6 (C-3a), 44.7 (C-4), 33.3 (C-6), 27.5 (C-5). ^{31}P-NMR (DMSO-d_6) δ ppm: 20.63. LCMS (m/z): 304 [M+·], 252, 127, 75. Anal. Calcd. for C15H17N2OPS: C, 59.20; H, 5.63; N, 9.20. Found: C, 59.12; H, 5.60; N, 9.18%.

(3aS)-1-(2,4,5-trichloroanilino)perhydro-2λ^5-pyrrolo[1,2-c][1,3,2]oxazaphosphole-2-thione (6c)

Yellow crystals. Yield: 75%. Mol.Wt.: 356. m.p.: 114-117°C. IR (KBr) ν cm^{-1}: 3308 (N-H), 1041 (P-O-C_{cyclic}), 1016 (P-N-$C_{substituted}$), 970 (P-N-C_{cyclic}), 791 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.82 (s, 1H, H-5^l), 7.13 (s, 1H, C-7^l), 6.38 (s, 1H, H-2^l), 3.25-3.35 (m, 2H, H-7), 3.02-3.17 (m, 1H, H-3a), 2.83-2.94 (m, 2H, H-4), 1.45-1.62 (m, 4H, H-5 & H-6). ^{31}P-NMR (DMSO-d6) δ ppm: 21.42. LCMS (m/z): 362 [M+6], 360 [M+4], 358[M+2], 356 [M+·], 293, 228, 190, 146. Anal. Calcd. for $C_{11}H_{12}Cl_3N_2OPS$: C, 36.94; H, 3.38; N, 7.83. Found: C, 36.80; H, 3.32; N, 7.78%.

(3aS)-1-(4-chloroanilino)perhydro-2λ^5-pyrrolo[1,2-c][1,3,2]oxazaphosphole-2-thione (6d)

Pale yellow crystals. Yield: 73%. Mol.Wt.: 288. m.p.: 148-151°C. IR (KBr) ν cm^{-1}: 3313 (N-H), 1038

(P-O-C_{cyclic}), 1020 (P-N-$C_{substituted}$), 965 (P-N-C_{cyclic}), 772 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.23 (m, 2H, H-3^l & H-5^l), 6.52 (m, 2H, H-2^l & H-6^l), 4.37 (s, 1H, H-7^l), 3.27-3.36 (m, 2H, H-7), 3.00-3.13 (m, 1H, H-3a), 2.81-2.90 (m, 2H, H-4), 1.44-1.64 (m, 4H, H-5 & H-6). ^{31}P-NMR (DMSO-d6) δ ppm: 20.35. LCMS (m/z): 290 [M+2], 288 [M+·], 203, 162, 138.

Ethyl (2S)-1-[(3aS)-2-thioxoperhydro-2λ5-pyrrolo [1,2-c][1,3,2]oxazaphosphol-1-yl] tetra- hydro-1H-2-pyrrolecarboxylate (6e)

Brown crystals. Yield: 79%. Mol.Wt.: 304. m.p.: 152-154°C. IR (KBr) ν cm^{-1}: 1724 (C=O), 1323 (O=C-O), 1035 (P-O-C_{cyclic}), 1014 (P-N-$C_{substituted}$), 954 (P-N-C_{cyclic}), 768 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.3 (q, 2H, C-9^l), 3.64-3.72 (m, 1H, H-5^l), 3.29-3.37 (m, 2H, H-7), 3.05-3.12 (m, 1H, H-3a), 2.80-2.87 (m, 4H, H-4 & H-2^l), 1.80-1.04 (m, 2H, H-4^l), 1.42-1.63 (m, 6H, H-5, H-6 & H-3^l), 1.4 (t, 3H, C-10^l). ^{31}P-NMR (DMSO-d_6) δ ppm: 21.40.

(3aS)-1-(1,3-benzithiazol-2-ylamino)perhydro-2λ5-pyrrolo[1,2-c][1,3,2]oxaza phosphole-2-thione (6f)

Brownish red crystals. Yield: 72%. Mol.Wt.: 311. m.p.: 135-138°C. IR (KBr) ν cm^{-1}: 3304 (N-H), 1658 (C=N), 1028 (P-N-$C_{substituted}$), 1024 (P-O-C_{cyclic}), 948 (P-N-C_{cyclic}), 778 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.20 (d, 1H, H-7^l), 7.38 (s, 1H, NH), 7.27 (d, 1H, H-6^l), 7.13 (s, 1H, H-4^l), 6.47 (s, 1H, H-5^l), 3.28-3.36 (m, 2H, H-7), 3.03-3.16 (m, 1H, H-3a), 2.42-2.87 (m, 2H, H-4), 1.62-1.98 (m, 4H, H-5 & H-6). ^{31}P-NMR (DMSO-d_6) δ ppm: 19.56.

(3aS)-1-[(6-methoxy-1,3-benzithiazol-2-yl)amino]perhydro-2λ5-pyrrolo[1,2-c][1,3,2]oxazapho-sphole-2-thione (6g)

Brown crystals. Yield: 80%. Mol.Wt.: 341. m.p.: 126-128°C. IR (KBr) ν cm^{-1}: 3312 (N-H), 1668 (C=N), 1029 (P-O-C_{cyclic}), 1023 (P-N-$C_{substituted}$), 957 (P-N-C_{cyclic}), 763 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.35 (s, 1H, H-7^l), 7.45 (s, 1H, NH), 7.28 (d, 1H, H-4^l), 7.13 (d, 1H, H-5^l), 3.65 (s, 3H, -CH_3), 3.43-3.60 (m, 2H, H-7), 3.04-3.13 (m, 1H, H-3a), 2.48-2.80 (m, 2H, H-4), 1.65-2.01 (m, 4H, H-5 & H-6). ^{13}C-NMR (DMSO-d_6) δ ppm: 175.7 (C-2^l), 158.1 (C-6^l), 149.4 (C-8^l), 129.4 (C-4^l), 126.7 (C-5^l), 122.1 (C-9^l), 106.7 (C-7^l), 64.1 (C-7), 59.7 (C-11^l), 45.1 (C-3a), 40.1 (C-4), 26.2 (C-6), 24.3 (C-5). ^{31}P-NMR (DMSO-d_6) δ ppm: 19.23. LCMS m/z : 341[M$^+$·], 322, 310, 293, 284, 170. Anal. Calcd. for: C, 45.74; H, 4.72; N, 12.31. Found: C, 45.68; H, 4.69; N, 12.24%.

(3aS)-1-[(6-nitro-1,3-benzithiazol-2-yl)amino] perhydro-2λ5-pyrrolo[1,2-c][1,3,2] oxazapho-sphole-2-thione (6h)

Yellow crystals. Yield: 68%. Mol.Wt.: 356. m.p.: 170-175°C. IR (KBr) ν cm^{-1}: 3334 (N-H), 1032 (P-O-Ccyclic), 1035 (P-N-$C_{substituted}$), 968 (P-N-C_{cyclic}), 790 (P=S), 1673 (C=N). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.30 (s, 1H, H-7^l), 7.44 (s, 1H, NH), 7.26 (d, 1H, H-5^l), 7.14 (d, 1H, H-4^l), 3.41-3.58 (m, 2H, H-7), 3.04-3.18 (m, 1H, H-3a), 2.45-2.75 (m, 2H, H-4), 1.64-1.98 (m, 4H, H-5 & H-6). ^{13}C-NMR (DMSO-d_6) δ ppm: 174.3 (C-2^l), 148.2 (C-8^l), 122.5 (C-5^l), 144.3 (C-6^l), 116.4 (C-7^l), 127.8 (C-4^l), 121.6 (C-9^l), 64.3 (C-7), 45.3 (C-3a), 40.7 (C-4), 26.1 (C-6), 27.9 (C-5). ^{31}P-NMR (DMSO-d_6) δ ppm: 19.82.

(3aS)-1-(1H-indolyl)perhydro-2λ5-pyrrolo[1,2-c][1,3,2]oxazaphosphole-2-thione (6i)

Brown crystals. Yield: 73%. Mol.Wt.: 278. m.p.: 133-136°C. IR (KBr) ν cm^{-1}: 1679 (C=N), 1022 (P-N-$C_{substituted}$), 1018 (P-O-C_{cyclic}), 964 (P-N-C_{cyclic}), 782 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.73 (d, 1H, H-4^l), 7.57 (d, 1H, H-7^l), 7.49 (d, 1H, H-2^l), 7.13 (m, 1H, H-6^l), 6.85 (m, 1H, H-5^l), 6.64 (d, 1H, H-3^l), 3.35-3.42 (m, 2H, H-7), 3.12-3.21 (m, 1H, H-3a), 2.86-2.97 (m, 2H, H-4), 1.49-1.68 (m, 4H, H-5 & H-6). LCMS m/z (%): 278 [M+•], 226, 146, 132. Anal. Calcd. for $C_{13}H_{15}N_2OPS$: C, 56.10; H, 5.43; N, 10.07. Found: C, 55.92; H, 5.35; N, 9.81%.

(3aS)-1-(1H-1,3-benzodiazol-1-yl)perhydro-2λ5-pyrrolo[1,2-c][1,3,2] oxazaphosphole-2-thione (6j)

Brown crystals. Yield: 81%. Mol.Wt.: 279. m.p.: 149-152°C. IR (KBr) ν cm^{-1}: 1665 (C=N), 1021 (P-N-$C_{substituted}$), 1016 (P-O-C_{cyclic}), 958 (P-N-C_{cyclic}), 787 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.16 (s, 1H, H-2^l), 7.75 (d, 1H, H-7^l), 7.73 (d, 1H, H-4^l), 7.28 (m, 1H, H-6^l), 7.24 (m, 1H, H-5^l), 3.33-3.41 (m, 2H,

H-7), 3.10-3.20 (m, 1H, H-3a), 2.84-2.93 (m, 2H, H-4), 1.45-1.65 (m, 4H, H-5 & H-6). ^{31}P-NMR (DMSO-d_6) δ ppm: 19.78. Anal. Calcd. for $C_{12}H_{14}N_3OPS$: C, 51.60; H, 5.05; N, 15.04. Found: C, 51.38; H, 4.98; N, 14.97%.

(3aS)-1-(1H-1,2,3-benzotriazol-1-yl)perhydro-2λ^5-pyrrolo[1,2-c][1,3,2]oxaza phosphole-2-thione (6k)

Brown crystals. Yield: 76%. Mol.Wt.: 280. m.p.: 140-143°C. IR (KBr) ν cm^{-1}: 1676 (C=N), 1023 (P-O-C_{cyclic}), 1013 (P-N-$C_{substituted}$), 963 (P-N-C_{cyclic}), 774 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.88 (d, 1H, H-7^l), 7.84 (d, 1H, H-4^l), 7.52 (m, 1H, H-6^l), 7.45 (m, 1H, H-5^l), 3.34-3.43 (m, 2H, H-7), 3.12-3.21 (m, 1H, H-3a), 2.83-2.95 (m, 2H, H-4), 1.43-1.65 (m, 4H, H-5 & H-6). ^{13}C-NMR (DMSO-d_6) δ ppm: 145.3 (C-4^1), 131.9 (C-9^l), 127.7 (C-7^l), 127.4 (C-6^1), 118.9 (C-8^l), 118.7 (C-5^1), 58.0 (C-7), 47.5 (C-3a), 44.3 (C-4), 33.4 (C-6), 27.3 (C-5). Anal. Calcd. for $C_{11}H_{13}N_4OPS$: C, 47.14; H, 4.68; N, 19.99. Found: C, 46.96; H, 4.57; N, 19.78%.

(3aS)-1-(1H-1,2,4-triazol-1-yl)perhydro-2λ^5-pyrrolo[1,2-c][1,3,2]oxazaphosphole-2-thione (6l)

Brown crystals. Yield: 77%. Mol.Wt.: 246. m.p.: 156-158°C. IR (KBr) ν cm^{-1}: 1658 (C=N), 1032 (P-O-C_{cyclic}), 1010 (P-N-$C_{substituted}$), 969 (P-N-C_{cyclic}), 785 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.35 (s, 1H, H-3^l), 8.22 (s, 1H, H-5^l), 3.24-3.35 (m, 2H, H-7), 3.03-3.16 (m, 1H, H-3a), 2.82-2.95 (m, 2H, H-4), 1.44-1.64 (m, 4H, H-5 & H-6). ^{13}C-NMR (DMSO-d_6): δ ppm 151.4 (C-3^l), 148.6 (C-5^l), 58.5 (C-7), 47.7 (C-3a), 44.3 (C-4), 33.1 (C-6), 27.8 (C-5). ^{31}P-NMR (DMSO-d_6) δ ppm: 19.89. Anal. Calcd. for $C_7H_{11}N_4O_2PS$: C, 34.15; H, 4.50; N, 22.75. Found: C, 33.97; H, 4.42; N, 22.57%.

1-[5-([(3aS)-2-thioxoperhydro-2λ^5-pyrrolo[1,2-c][1,3,2]oxazaphosphol-1-yl]oxymethyl)-4-azidotetrahydro-2-furanyl]-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidine dione (6m)

Brown crystals. Yield: 65%. Mol.Wt.: 428. m.p.: 182-185°C. IR (KBr) ν cm^{-1}: 3317 (N-H), 1688 (C=O), 1092 (P-O-$C_{substituted}$), 1029 (P-O-C_{cyclic}), 958 (P-N-C_{cyclic}), 774 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.60 (s, 1H, H-2^l), 7.45 (s, 1H, H-5^l), 5.85 (m, 1H, H-7^l), 3.83-3.92 (m, 1H, H-10^l), 3.78-3.91 (m, 2H, H-15^l), 3.23-3.31 (m, 2H, H-7), 3.01-3.14 (m, 1H, H-3a), 2.45 (s, 3H, H-14^l), 2.35-2.48 (m, 2H, H-8^l), 2.80-2.89 (m, 2H, H-4), 1.92-2.05 (m, 1H, H-9^l), 1.42-1.61 (m, 4H, H-5 & H-6). ^{13}C-NMR (DMSO-d_6) δ ppm: 163.7 (C-3^l), 150.8 (C-1^l), 136.2 (C-5^l), 110.8 (C-4^l), 86.5 (C-7^l), 84.3 (C-10^l), 57.4 (C-7), 54.9 (C-15^l), 49.6 (C-9^l), 46.4 (C-3a), 43.9 (C-4), 38.7 (C-8^l), 32.8 (C-6), 27.2 (C-5), 12.8 (C-14^l). ^{31}P-NMR (DMSO-d_6) δ ppm: 20.42. LCMS m/z : 428 [M+·], 376, 285, 190, 146. Anal. Calcd. for $C_{15}H_{21}N_6O_5PS$: C, 42.05; H, 4.94; N, 19.62. Found: C, 41.89; H, 4.83; N, 19.39%.

4-amino-1-[2-([(3aS)-2-thioxoperhydro-2λ^5-pyrrolo[1,2-c][1,3,2]oxazaphosphol-1-yl]oxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydro-2-pyrimidinone (6n)

Brown crystals. Yield: 67%. Mol.Wt.: 390. m.p.: 170-173°C. IR (KBr) ν cm^{-1}: 3325 (N-H), 1692 (C=O), 1688 (C=N), 1105 (P-O-$C_{substituted}$), 1035 (P-O-C_{cyclic}), 963 (P-N-C_{cyclic}), 781 (P=S). ^{1}H-NMR (DMSO-d_6) δ ppm: 9.16 (s, 1H, H-4^l), 6.69 (s, 2H, H-14^l), 5.25 (m, 1H, H-7^l), 5.20-5.29 (m, 2H, H-11^l), 4.57 (s, 1H, H-5^l), 3.91-4.16 (m, 1H, H-12^l), 3.87-3.94 (m, 2H, H-9^l), 3.23-3.31 (m, 2H, H-7), 3.01-3.14 (m, 1H, H-3a), 2.80-2.89 (m, 2H, H-4), 1.42-1.61 (m, 4H, H-5 & H-6). ^{13}C-NMR (DMSO-d_6) δ ppm: 165.7 (C-6^l), 155.4 (C-2^l), 143.8 (C-4^l), 94.5 (C-5^l), 87.9 (C-7^l), 80.5 (C-9^l), 63.7 (C-12^l), 57.4 (C-7), 46.4 (C-3a), 43.9 (C-4), 32.8 (C-6), 29.7 (C-11^l), 27.2 (C-5). ^{31}P-NMR (DMSO-d_6) δ ppm: 20.83. LCMS m/z: 390 [M+·] , 190, 146. Anal. Calcd. for $C_{13}H_{19}N_4O_4PS_2$: C, 39.99; H, 4.91; N, 14.35. Found: C, 39.78; H, 4.83; N, 14.20%.

PHARMACOLOGY

Antioxidant activity

DPPH radical-scavenging activity

The DPPH radical scavenging activity was measured in a reaction mixture containing 1 mM DPPH radical solution 0.1 mL, 99% ethanol 0.8 mL, and 0.1 mL of each one of the studied component prepared by dissolving the compound in methanol. The solution was rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the de-

crease in absorbance at 517 nm. The antioxidant activity of test compounds was expressed as IC_{50}, which was defined as the concentrations of test compounds required for inhibition of the formation of DPPH radicals by 50%.

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{1 – absorbance of sample at 517nm}}{\text{absorbance of control at 517nm}} \times 100$$

The antioxidant activity evaluation with the 1,1-diphenyl-2-picryl-hydrazyl (DPPH), radical-scavenging assay was carried out[18] and the results are tabulated in TABLE 1.

Superoxide radical scavenging activity

Superoxide radicals were determined using spectrophotometric measurement of the effects of various concentrations of test compounds on the reduction of nitroblue tetrazolium (NBT), according to a previously described procedure[19]. Superoxide radicals were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system. The non-enzymatic generation of superoxide radicals was measured in reaction mixtures containing various concentrations of test compounds, PMS (15 lM), NADH (73 lM), and NBT (50 lM) in phosphate buffer (20 mM, pH 7.4). After incubation for 5 min at ambient temperature, the color was read at 560 nm against blank samples. The superoxide radical-scavenging activity was expressed as the IC_{50} value. Superoxide dismutase enzyme (SOD) was used as a positive control.

$$\text{superoxide radical scavenging activity (\%)} = \frac{\text{absorbance of control – absorbance of sample}}{\text{absorbance of control}} \times 100$$

Reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$) and peroxyl radicals ($ROO^{\cdot}$) are produced as a part of normal metabolic processes[20]. The compounds **(6a-n)** showed high antioxidant activity by scavenging the free radicals and superoxide radicals.

Antimicrobial activity

Antimicrobial activity was determined against bacterial and fungal pathogens by the paper disc diffusion assay. Bacterial strains belong to both Gram +ve and –ve were used in this study. Gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria *Escherichia coli*, *Klebsiella pneumonia* were maintained on nutrient agar at 37°C and overnight cultures were prepared in nutrient broth. The cultures were diluted with sterilized saline to bring the final inoculum size of approximately 10^5-10^6 CFU/mL. The compounds were diluted in DMSO for biological assays.

TABLE 1 : Antioxidant activities of the title compounds (6a-n)

Compound	DPPH scavenging (%)	Superoxide scavenging (%)
6a	70.47±1.34	65.12±1.55
6b	65.02±1.45	61.07±1.73
6c	72.13±1.21	67.45±1.64
6d	65.84±1.08	60.93±1.86
6e	71.34±1.36	69.54±1.57
6f	64.87±1.15	61.64±1.09
6g	74.68±1.12	72.19±1.37
6h	64.94±1.73	61.08±1.88
6i	72.48±1.84	69.97±1.73
6j	65.69±1.58	60.96±1.01
6k	65.22±1.06	61.87±1.12
6l	66.81±1.26	61.67±1.29
6m	78.04±1.89	72.57±1.91
6n	80.21±1.72	74.33±1.54
Vitamin C	83.42 ± 1.65	78.51± 1.43

Antimicrobial activity assay

The test compounds were introduced onto the disc and then allowed to dry to completely saturate the disc. Then the disc was introduced onto the upper layer of the medium with the bacteria. The Petri dishes were incubated overnight at 37°C for 24 hr and the fungal cultures were prepared in potato dextrose broth and incubated at 25°C for 5-7 days. These antimicrobial activities were assessed based on measurement of the diameter of the clear zone around the paper disc. The experiments were carried out in triplicates. Petri were examined for zone of inhibition around each disc and average results were recorded. The results were compared with the activity of the standard antibiotic Penicillin (30 µg/mL). The antibacterial activity was evaluated and the results are presented in TABLE 2.

TABLE 2 : Antibacterial activity of title compounds

Compound	MIC Zone of Inhibition(mm)			
	B. subtilis	*S.aureus*	*E.coli*	*K. pneumonia*
6a	10.3	9.8	9.7	10.0
6b	9.0	10.0	11.0	9.8
6c	10.5	10.0	12.1	11.6
6d	10.0	9.5	9.4	9.7
6e	11.8	10.2	11.1	10.0
6f	10.4	10.2	10.7	9.4
6g	13.7	13.1	12.8	11.9
6h	10.7	10.5	10.8	10.5
6i	13.0	12.0	12.5	11.0
6j	10.3	10.0	10.5	10.4
6k	9.2	9.2	9.8	9.6
6l	9.5	9.8	9.0	9.3
6m	14.1	13.5	13.3	11.5
6n	14.3	14.0	15.7	12.6
Penicillin(30 µg/mL)	16.7	17.2	16.4	18.2

TABLE 3 : Antifungal activity of title compounds

Compound	MIC Zone of Inhibition(mm)	
	A. niger	*C. albicans*
6a	9.7	9.5
6b	8.5	8.2
6c	9.8	9.5
6d	8.3	8.6
6e	9.4	9.2
6f	8.2	8.4
6g	10.5	9.7
6h	8.8	9.1
6i	9.1	9.3
6j	9.0	9.1
6k	8.2	7.7
6l	10.3	9.8
6m	10.7	10.2
6n	10.9	10.2
Amphotericin B (50 µg/mL)	13.2	12.5

Minimum inhibitory concentration (MIC) of compounds

MIC of the samples **(6a-n)** was determined by the broth dilution method using the serially diluted compounds as described with plant material[21]. The media containing compounds were diluted with distilled water to give concentrations ranged from 40 to 0.1mg/mL. 100 µL of the diluted fungal culture, 0.6mL of potato dextrose broth, and 0.6mL of the compound were mixed well in a test tube. The mixture was then incubated at 25°C for 72 h to determine the minimal concentration at which growth of fungal cells was fully inhibited. The antifungal activity was evaluated and the results are tabulated in TABLE 3.

These solutions containing 10^6 cells/mL were added to each Whatmann No.1 filter paper disc (6 mm diameter) and DMSO was used as the control. The compounds **(6a-n)** was taken with the concentration of 100 µg/mL and was evaluated by disc diffusion method for antimicrobial activity. The compounds **(6g)**, **(6l)**, **(6m)** and **(6n)** showed higher activity against both the Gram positive and negative bacteria and fungi when compared to the standards *Penicillin* and *Amphotericin B* respectively. The compounds **(6a)**, **(6c)**, **(6e)**, **(6i)**, and **(6j)** exhibited moderate activity against the bacterial and fungal cultures when compared to the other compounds. Majority of the compounds exhibited promising antimicrobial activity.

CONCLUSION

The pharmacological activity of the compounds with amine substituents, zidovudine and lamivudine bearing electron-donating methoxy substituents, electron-withdrawing nitro substituents and chloro substituents and ester containg groups relative to the N–H bond are able to establish some structure-antioxidant-activity relationship.

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