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Synthesis and anti-microbial activity of novel mannich bases containing 2- phenoxy-1, 3, 2-dioxa phospholanes and Indole systems

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ABSTRACT

New mannich bases of 2-phenoxy-1,3,2-dioxaphospholanes derivatives (4R, 5R) - N'4, N'5-bis-(2-oxo-1-(piperidin-1-ylmethyl) / (morpholino methyl) / (4-methyl piperazin-1-ylmethyl) indolin-3-ylidene) - 2-(4-substituted phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide ***6(a-g)*** *were prepared by the condensation reaction between (4R, 5R) -2-(4-substituted phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide* ***3(a-e)*** *with Isatin* ***(4)*** *yielded the corresponding (4R, 5R) - N'4, N'5-bis-(2-oxo indolin-3-ylidene) - 2-(4-substituted phenoxy)-1, 3, 2-Dioxaphospholane-4, 5-dicarbohydrazide-2-oxide* ***5(a-e).*** *This was allowed to undergo the Mannich reaction with different Secondary Amines namely: piperidine, morpholine and N-methyl piperazine in the presence of formaldehyde in DMF to give corresponding hydrazides* ***6(a-g).*** *The structure of these newly synthesized compounds was characterized by ^{1}H-NMR, Mass, IR, C^{13}-NMR and P^{31}-NMR Spectral data.These newly synthesized compounds* ***6(a-g)*** *were screened for their antibacterial and antifungal activity.*

Keywords: Dioxaphospholanes, Isatin, Mannich bases, Antibacterial and Antifungal activity.

INTRODUCTION

Tartaric acid and its derivatives are widely available, frequently used and inexpensive chiral compounds which play an important role in the construction of numerous chiral organic molecules [1]. Tartaric acid and its derivatives are known to show a wide variety of biological applications in the agro chemical [2] and medical fields [3], industrial applications in the field to build up organo-metalic complexes [4,5]. These are also used as antioxidants, muscle toxins, preservatives after fermentation [6]. Two chiral carbons with hydroxyl substituent make them as excellent starting materials in organic synthesis.

A good deal of importance is given to 1, 3, 2-Dioxaphosphorinane and Dioxaphospholane derivatives in the field of Organophosphorous heterocyclic chemistry due to their unique stereochemical futures and diverse potential biological applications [7, 8]. Indole systemsand related heterocyclic compounds [9] containing phosphorus moiety possess various types of biological activities. It is due to their wide use in medicinal chemistry and some of them possess antiviral, anti tumor and antihypertensive activities. The anti bacterial activity of Mannich bases has been well established [10-13]. In view of these observations, it appeared of interest to synthesis some novel double headed active 1, 3, 2-Dioxaphospholane derivatives of tartaric acid moiety containing indole based mannich bases.

EXPERIMENTAL SECTION

All the chemicals used in the present investigation were purchased from Sigma-Aldrich Chemicals Company, Inc. USA. and used without further purification. TLC was performed on alluminium sheet of silica gel $60F_{254,}$ E-Merk, Germany using iodine as visualizing agent. Melting points were determined in open capillary tubes on Mel-Temp apparatus and are uncorrected. Column chromatography was performed on silica gel with different solvent systems as eluents to afford the pure compound. The IR Spectra were recorded as KBr pellets on Perkin-Elmer 1000 unit, Instrument. All H^1 and C^{13}-NMR spectra were recorded on a Varian XL-300 spectrometer operating at 300MHz for H^1-NMR and 75.46 MHz for C^{13}-NMR. P^{31}-NMR spectra were recorded on a Varian XL-spectrometer operating at 161.89MHz. The compounds were dissolved in DMSO-d6 and Chemical shifts were referenced to TMS (H^1 and C^{13}-NMR) and 85% H_3PO_4 (P^{31}-NMR). Mass spectral data was recorded on FAB-MS instrument at 70ev with direct inlet system. Elemental analyses were recorded on a Carlo Erba 1108 Elemental Analyser, Central Drug Research Institute, Lucknow, India.

Preparation of Intermediates:

4- substituted phenyl phosphorodichloridates :

Phosphorous oxychloride (0.1mole) in dry benzene (60ml) was taken into a three necked flask (500ml) equipped with dropping funnel and reflux condenser fitted with a calcium chloride guard tube. The flask was heated and stirred by means of hot plate cum magnetic stirrer. Dry trimethyl amine (0.1mol) and dry benzene (50ml) were added into the flask slowly while stirring. To this mixture, freshly distilled phenol (0.1mol) in dry benzene (60ml) was added drop wise through the dropping funnel. The addition took about 30 minutes and the whole reaction mixture was refluxed with vigorous stirring for 10 hours. The reaction mixture was cooled and the solid triethylamine hydrochloride was filtered off. The solvent from the filtrate was removed under reduced pressure in a rotaevaporator. The dark brown liquid remained was subjected to fractional distillation and the major product distilling at 118-124^{0}C / 11mm was collected as colorless glassy viscous liquid [14].

Other substituted phenyl phosphorodichloridates **1(a-e)** were prepared by the same procedure [15-18] by treating equimolar quantities of phosphorousoxychloride and respective substituted phenols in benzene in the presence of triethylamine.

(4R, 5R) -Diethyl 2 - (4-substituted phenoxy) - 1, 3, 2 - dioxa phospholane-4, 5-dicarboxylate - 2-oxide 2(a-e):

A solution of phenyl phosphorodichloridate (0.002mole) in 25ml of dry toluene was added drop wise over a period of twenty minutes to a stirred solution of diethyl-2s, 3s-dihydroxy butane dioate (0,002mole) and tri ethyl amine (0.004mole) in 30ml of dry toluene and 10ml of tetra hydro furan. After completion of the addition the addition temperature of the reaction mixture was slowly raised to room temperature and stirred for 2 hours. Latter the reaction mixture was heated to 50-60^{0}C and maintained for 4 hours with stirring. The completion of the reaction was monitored by TLC analysis [19]. Triethyl amine hydrochloride was filtered off from mixture and solvent was removed under reduced pressure in rotaevaporator. The crude product 2(a) was obtained as a brown gummy solid. Similar procedure [20] was adopted to synthesize 2(b-e) from 1(b-e).

Most of the crude solids obtained were highly unstable and have turned into gummy and viscous semi solids when exposed to air. Fairly pure and stable products are obtained from these gums with 2-propanol, sometimes small amount of n-hexane or petroleum ether (60-80^{0}C) was added to the 2-propanol solution of these compounds to induce recrystallization.

(4R, 5R) -2-(4-substituted phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide 3(a-e):

A solution of 2(a) (0.01mol) and hydrazine hydrate (0.03mol) in absolute ethanol- tetra hydro furan (1:1) mixture was refluxed for 5 hours. The course of progress of the reaction was monitored by TLC. After completion of the reaction the solvent was removed in rotaevaporator. The crude gummy solid was recrystallized from 2-propanol and petroleum ether (60-80^{0}C) solvent mixture to afford (4R, 5R) -2-phenoxy-1, 3, 2-dioxa phospholane- 4, 5-dicarbohydrazide-2-oxide 3(a). Similar procedure [21, 22] was adopted to synthesise 3(b-e) from 2(b-e). The compounds thus obtained were characterized by their elemental analysis and spectral data (IR, H^1-NMR, P^{31}-NMR).

Physical, analytical and spectral data for the compounds 3(a-e):

(4R, 5R) -2-(phenoxy)-1, 3, 2-dioxa phospholane- 4, 5-dicarbohydrazide-2-oxide 3(a):
Yield: 50%; M.p: 106-108ºC; IR (KBr): 3496, 3413 cm^{-1} (-NH_2), 3205cm^{-1} (-NH), 1690 cm^{-1} (>C=O), 1258 cm^{-1} (P=O), 894 cm^{-1} and 1032cm^{-1} (P-O-C $_{aliphatic}$), 954 cm^{-1} and 1196 cm^{-1} (P-O-C $_{aromatic}$); ^{1}HNMR (300MHz, DMSO-*d6*): δ 4.27(S, 4H, -NH_2), 8.95(S, 2H, -NH-), 4.49(s, 2H, -CH- of dioxaphospholane ring), 7.18-7.28(m, 5H, C_6H_5) ; ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.36. Anal. Calcd.(%) for $C_{10}H_{13}N_4O_6P$: C 37.98, H 4.14, N 17.72; Found: C 37.87, H 4.09, N 17.65.

(4R, 5R) -2-(4-methyl phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide 3(b):
Yield: 55%; M.p: 118-120ºC; IR (KBr): 3498, 3412 cm^{-1} (-NH_2), 3206cm^{-1} (-NH), 1687 cm^{-1} (>C=O), 1279 cm^{-1} (P=O), 893 cm^{-1} and 1019cm^{-1} (P-O-C $_{aliphatic}$), 960 cm^{-1} and 1190 cm^{-1} (P-O-C $_{aromatic}$); ^{1}HNMR (300MHz, DMSO-*d6*): δ 4.30(S, 4H, -NH_2), 8.92(S, 2H, -NH-), 4.47(s, 2H,CH- of dioxaphospholane ring), 6.83-7.06(m, 4H, C_6H_4), 3.15(S, 3H, Ar-CH_3); ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.46 ppm. Anal. Calcd.(%) for $C_{11}H_{15}N_4O_6P$: C 40.01, H 4.53, N 16.97; Found: C 39.98, H 4.47, N 16.91.

(4R, 5R) -2-(4-chloro phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide 3(c):
Yield: 60%; M.p: 132-134ºC; IR (KBr): 3498, 3414 cm^{-1} (-NH_2), 3209cm^{-1} (-NH), 1693 cm^{-1} (>C=O), 1254 cm^{-1} (P=O), 905 cm^{-1} and 1025cm^{-1} (P-O-C $_{aliphatic}$), 952 cm^{-1} and 1192 cm^{-1} (P-O-C $_{aromatic}$); ^{1}HNMR (300MHz, DMSO-*d6*): δ 4.32(S, 4H, -NH_2), 9.10(S, 2H, -NH-), 4.47(s, 2H, -CH- of dioxaphospholane ring), 6.89-7. 32(m, 4H, C_6H_4); ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.59. Anal. Calcd.(%) for $C_{10}H_{12}N_4O_6PCl$: C 34.25, H 3.45, N 15.98; Found: C 34.21, H 3.39, N 15.86.

(4R, 5R) -2-(4-bromo phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide 3(d):
Yield: 55%; M.p: 120-122ºC; IR (KBr): 3498, 3414 cm^{-1} (-NH_2), 3212cm^{-1} (-NH), 1694 cm^{-1} (>C=O), 1260 cm^{-1} (P=O), 901 cm^{-1} and 1029cm^{-1} (P-O-C $_{aliphatic}$), 954 cm^{-1} and 1194 cm^{-1} (P-O-C $_{aromatic}$).; ^{1}HNMR (300MHz, DMSO-*d6*): 4.35(S, 4H, -NH_2), 9.14(S, 2H, -NH-), 4.47(s, 2H, -CH- of dioxaphospholane ring), 6.84-7.43(m, 4H, C_6H_4); ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.04. Anal. Calcd.(%) for $C_{10}H_{12}N_4O_6PBr$: C 30.40, H 3.06, N 14.18; Found: C 30.34, H 3.00, N 14.09.

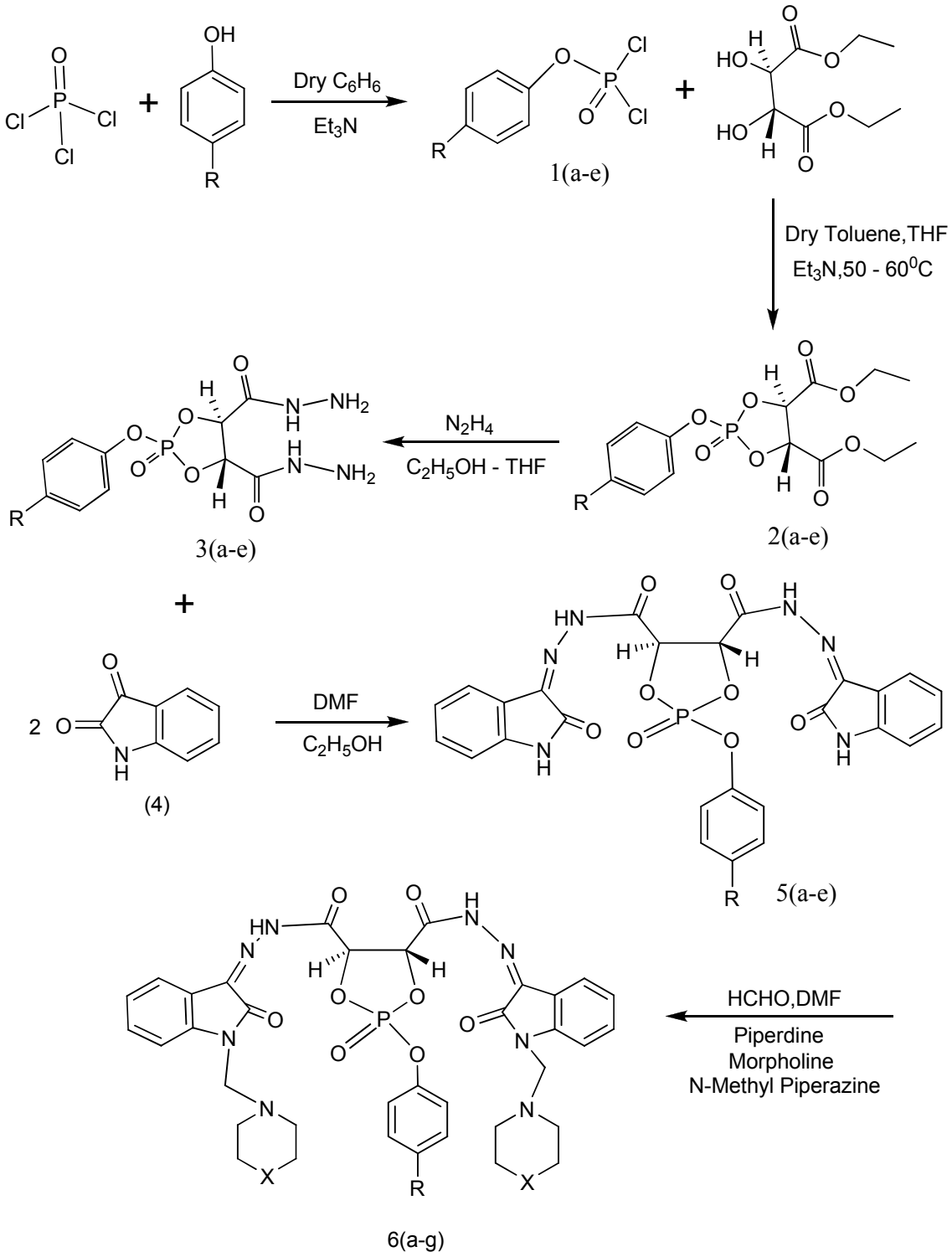
(4R, 5R) -2-(4-nitro phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide 3(e):
Yield: 50%; M.p: 98-100ºC; IR (KBr 3498, 3415 cm^{-1} (-NH_2), 3218cm^{-1} (-NH), 1689 cm^{-1} (>C=O), 1268 cm^{-1} (P=O), 960 cm^{-1} and 1035cm^{-1} (P-O-C $_{aliphatic}$), 960 cm^{-1} and 1203 cm^{-1} (P-O-C $_{aromatic}$).; ^{1}HNMR (300MHz, DMSO-*d6*): δ 4.35(S, 4H, -NH_2), 9.16(S, 2H, -NH-), 4.47(s, 2H, -CH- of dioxaphospholane ring), 7.24-8.09(m, 4H, C_6H_4); ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.35. Anal. Calcd.(%) for $C_{10}H_{12}N_5O_8P$: C 33.21, H 3.35, N 19.39; Found: C 33.21, H 3.28, N 19.29.

RESULTS AND DISCUSSION

Typical procedure for the synthesis of (4R, 5R) - N'4, N'5-bis-(2-oxo-1-(piperidin-1-yl methyl)/ (morpholino methyl) / (4-methyl piperazin-1-ylmethyl) indolin-3-ylidene) - 2-(4-substituted phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide 6(a-g):

A mixture of (0.02mol) of Isatin **(4)** [23] and the corresponding hydrazide (3a) (0.01 mol) were dissolved in absolute ethanol – DMF (2:1) solvent mixture. The reaction mixture was refluxed for 1-4 hours and then kept at room temperature overnight. The course of the progress of the reaction was monitored by TLC. After completion of the reaction the solvent was removed in rotaevaporator. The solid was recrystallized from 2-propanol and petroleum ether (60-80^{0}C) solvent mixture to afford (4R, 5R) - N'4, N'5-bis-(2-oxo indolin-3-ylidene) – 2 – phenoxy - 1, 3, 2 - Dioxaphospholane-4, 5-dicarbohydrazide-2-oxide 5(a). Similar procedure [24] was adopted to synthesize 5(b-e) from 3(b-e).

A mixture of (5) (0.1mol), piperidine (0.3 mol) and DMF were stirred to obtain a clear solution. To this solution, HCHO (0.05 mol) and DMF were added in ice-cold condition and stirred for 6 hours in an ice-bath and left overnight at room temperature. The obtained solid was isolated and crystallized from 2-proponal and petroleum ether (60-80^{0}C) solvent mixture to give Compound **(6a).** The reaction procedure leading to **(6a)** was then extended to the syntheses [25] of **(6b-g)**.



SCHEME-I

Compounds	6a	6b	6c	6d	6e	6f	6g
R	4-H	4-CH_3	4-Cl	4-Br	4-NO_2	4-H	4-H
X	CH_2	CH_2	CH_2	CH_2	CH_2	O	N-CH_3

Similar treatment of hydrazone **(5a)** with Morpholine / N-methyl piperazine in presence of formaldehyde in DMF at room temperature afforded the respective hydrazide (4R, 5R) - N'4, N'5-bis-(2-oxo- (morpholino methyl) / 1 -(4-methyl piperazin-1-ylmethyl) indolin-3-ylidene)- 2- phenoxy-1, 3, 2-dioxa phospholane-4,5-dicarbohydrazide-2-oxide **(6f /6g).** These reactions are summarized in the scheme-I. The purity of the compounds was monitored by TLC.

The structures of these newly synthesized compounds **(6a-g)** were established [26, 27] based on their elemental analysis and spectral data (IR, H^1-NMR, P^{31}-NMR, C^{13}-NMR and Mass).

Physical, analytical and spectral data for the compounds 6(a-g):

(4R, 5R) - N'4, N'5-bis- (2 - oxo -1- (piperidin-1-yl methyl) indolin-3-ylidene) - 2-(phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide (6a):

Yield: 60%; M.p: 133-135ºC; IR (KBr): 1760 (Indole C = O), 1652 (Carbonyl of CO – NH) and 2947cm^{-1} (CH_2), 1254 cm^{-1} (P=O), 897 cm^{-1}and 1030 cm^{-1} (P-O-$C_{aliphatic}$), 952 cm^{-1}and 1194 cm^{-1} (P-O-$C_{aromtic}$); ^{1}HNMR (300MHz, DMSO-*d6*): δ 1.53–1.59 (m, 12H $(CH_2)_3$ of two piperidine rings), 2.45 (t, 8H, –CH_2–N–CH_2 of two piperidine rings), 4.03(s, 4H, –N–CH_2–N–), 4.47(s, 2H,dioxaphospholane ring), 8.90 (s, 2H, CO-NH), 7.18-7.86 (m, 13H, for C_6H_5 ring ,two C_6H_4 rings); C^{13}-NMR (75.46 MHz, DMSO-*d6*): δ 150.3(C-1),115.9(C-2&6),130.2(C-3&5), 121.4(C-4) of ringA, 93.1(C-4' and C-5'), 177(CO-NH attached to Dioxa phospholane ring) of ring B, 163.5(C-2"), 132.8(C-3"), 129.4(C-4"), 124.5(C-5"), 131.3(C-6"), 121.7(C-7"), 147.4(C-8"), 117.8(C-9"), 70.4(N-CH_2-N) of ring C&D and 52.0(C-2&6), 25.6(C-3&5), 25.9(C-4) of ring E. ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.60. Anal. Calcd.(%) for $C_{38}H_{41}N_8O_8P$: C 59.37, H 5.38, N 14.58; Found: C 59.28, H 5.48, N 14.47.

(4R, 5R) - N'4, N'5-bis- (2 - oxo -1- (piperidin-1-yl methyl) indolin-3-ylidene) - 2-(4-methyl phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide (6b):

Yield: 55%; M.p: 148-150ºC; IR (KBr): 1758 (Indole C = O), 1655 (Carbonyl of CO – NH) and 2933cm^{-1} (CH_2), 1259 cm^{-1} (P=O), 895 cm^{-1}and 1021 cm^{-1} (P-O-$C_{aliphatic}$), 954 cm^{-1}and 1196 cm^{-1} (P-O-$C_{aromtic}$); ^{1}HNMR (300MHz, DMSO-*d6*): 1.53–1.59 (m, 12H $(CH_2)_3$ of two piperidine rings), 2.45 (t, 8H, –CH_2–N–CH_2 of two piperidine rings), 4.03 (s, 4H, –N–CH_2–N–), 4.47(s,2H,dioxaphospholane ring), 8.85 (s, 2H, CO-NH), 6.83-7.86 (m, 12H, for three C_6H_4 rings), 3.15(S, 3H, Ar-CH_3); C^{13}-NMR (75.46 MHz, DMSO-*d6*): δ 147.3(C-1), 115.8(C-2&6),130.5(C-3&5), 131.0(C-4), 24.3(-CH_3) of ringA, 93.1(C-4' and C-5'), 177(CO-NH attached to Dioxa phospholane ring) of ring B, 163.5(C-2"), 132.8(C-3"), 129.4(C-4"), 124.5(C-5"), 131.3(C-6"), 121.7(C-7"), 147.4(C-8"), 117.8(C-9"), 70.4(N-CH_2-N) of ring C&D and 52.0(C-2&6), 25.6(C-3&5), 25.9(C-4) of ring E. ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.51. Anal. Calcd.(%) for $C_{39}H_{43}N_8O_8P$: C 59.84, H 5.54, N 14.31; Found: C 59.75, H 5.48, N 14.27.

(4R, 5R) - N'4, N'5-bis- (2 - oxo -1- (piperidin-1-yl methyl) indolin-3-ylidene) - 2-(4-chloro phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide (6c):

Yield: 50%; M.p: 163-165ºC; IR (KBr): 1759 (Indole C = O), 1658 (Carbonyl of CO – NH) and 2920cm^{-1} (CH_2), 1257 cm^{-1} (P=O), 908 cm^{-1}and 1027 cm^{-1} (P-O-$C_{aliphatic}$),959 cm^{-1}and 1195 cm^{-1} (P-O-$C_{aromtic}$); ^{1}HNMR (300MHz, DMSO-*d6*): 1.53–1.59 (m, 12H $(CH_2)_3$ of two piperidine rings), 2.45 (t, 8H, –CH_2–N–CH_2 of two piperidine rings), 4.03 (s, 4H, –N–CH_2–N–), 4.47(s,2H,dioxaphospholane ring), 8.95 (s, 2H, CO-NH), 6.89-7.86 (m, 12H, for three C_6H_4 rings); C^{13}-NMR (75.46 MHz, DMSO-*d6*): δ 148.4(C-1), 117.3(C-2&6),130.3(C-3&5), 126.9(C-4) of ringA, 93.1(C-4' and C-5'), 177(CO-NH attached to Dioxa phospholane ring) of ring B, 163.5(C-2"), 132.8(C-3"), 129.4(C-4"), 124.5(C-5"), 131.3(C-6"), 121.7(C-7"), 147.4(C-8"), 117.8(C-9"), 70.4(N-CH_2-N) of ring C&D and 52.0(C-2&6), 25.6(C-3&5), 25.9(C-4) of ring E. ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.63. Anal. Calcd.(%) for $C_{38}H_{40}N_8O_8PCl$: C 56.82, H 5.02, N 13.95; Found: C 56.73, H 4.95, N 13.86.

(4R, 5R) - N'4, N'5-bis- (2 - oxo -1- (piperidin-1-yl methyl) indolin-3-ylidene) - 2-(4-bromo phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide (6d):

Yield: 50%; M.p: 157-158ºC; IR (KBr): 1762 (Indole C = O), 1660 (Carbonyl of CO – NH) and 2929cm^{-1} (CH_2), 1260 cm^{-1} (P=O), 903 cm^{-1}and 1029 cm^{-1} (P-O-$C_{aliphatic}$), 955 cm^{-1}and 1194 cm^{-1} (P-O-$C_{aromtic}$).; ^{1}HNMR (300MHz, DMSO-*d6*): δ 1.53–1.59 (m, 12H $(CH_2)_3$ of two piperidine rings), 2.45 (t, 8H, –CH_2–N–CH_2 of two piperidine rings), 4.03 (s, 4H, –N–CH_2–N–), 4.47(s,2H,dioxaphospholane ring), 8.92 (s, 2H, CO-NH), 6.84-7.86 (m, 12H, for three C_6H_4 rings); C^{13}-NMR (75.46 MHz, DMSO-*d6*): δ 149.3(C-1),118.1(C-2&6),133.2(C-3&5), 115.7(C-4) of ringA, 92.1(C-4' and C-5'), 176.5(CO-NH attached to Dioxa phospholane ring) of ring B, 163.1(C-2"), 131.8(C-3"), 129.1(C-4"), 125.1(C-5"), 132.3(C-6"), 121.9(C-7"), 147.8(C-8"), 117.5(C-9"), 70.3(N-CH_2-N) of ring C&D and 51.3(C-2&6), 24.9(C-3&5), 25.5(C-4) of ring E. ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.24. Anal. Calcd.(%) for $C_{38}H_{40}N_8O_8PBr$: C 53.84, H 4.76, N 13.22; Found: C 53.77, H 4.67, N 13.14.

(4R, 5R) - N'4, N'5-bis- (2 - oxo -1- (piperidin-1-yl methyl) indolin-3-ylidene) - 2-(4-nitro phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide (6e):
Yield: 45%; M.p: 127-129ºC; IR (KBr): 1763 (Indole C = O), 1665 (Carbonyl of CO – NH) and 2920cm^{-1} (CH_2), 1264 cm^{-1} (P=O), 902 cm^{-1}and 1032 cm^{-1} (P-O-$C_{aliphatic}$), 957 cm^{-1}and 1196 cm^{-1} (P-O-$C_{aromtic}$); ^{1}HNMR (300MHz, DMSO-*d6*): δ 1.53–1.59 (m, 12H $(CH_2)_3$ of two piperidine rings), 2.45 (t, 8H, –CH_2–N–CH_2 of two piperidine rings), 4.03 (s, 4H, –N–CH_2–N–), 4.47(s,2H,dioxaphospholane ring), 8.96 (s, 2H, CO-NH), 7.24-8.09 (m, 12H, for three C_6H_4 rings); C^{13}-NMR (75.46 MHz, DMSO-*d6*): δ 156.4(C-1),118.1(C-2&6),122.5(C-3&5), 141.0(C-4) of ringA, 93.5(C-4' and C-5'), 176.2(CO-NH attached to Dioxa phospholane ring) of ring B, 165.5(C-2"), 130.3(C-3"), 128.9(C-4"), 125.3(C-5"), 132.1(C-6"), 120.9(C-7"), 147.1(C-8"), 117.3(C-9"), 71.0(N-CH_2-N) of ring C&D and 51.3(C-2&6), 24.9(C-3&5), 25.7(C-4) of ring E. ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.35. Anal. Calcd.(%) for $C_{38}H_{40}N_9O_{10}P$: C56.09, H 4.95, N 15.49; Found: C 55.99, H 4.88, N 15.49.

(4R, 5R) - N'4, N'5-bis-(2- oxo - (morpholino methyl) indolin-3-ylidene) - 2-(phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide (6f):
Yield: 50%; M.p: 173-175ºC; IR (KBr): 1756 (Indole C = O), 1654 (Carbonyl of CO – NH) and 2925cm^{-1} (CH_2), 1258 cm^{-1} (P=O), 899 cm^{-1}and 1025 cm^{-1} (P-O-$C_{aliphatic}$), 956 cm^{-1}and 1195 cm^{-1} (P-O-$C_{aromtic}$); ^{1}HNMR (300MHz, DMSO-*d6*): δ 3.65 (t, 8H, –CH_2–O–CH_2 of two morpholine rings), 2.50 (t, 8H, –CH_2–N–CH_2 of two morpholine rings), 4.03 (s, 4H, –N–CH_2–N–), 4.47(s,2H,dioxaphospholane ring), 8.86 (s, 2H, CO-NH), 7.18-7.86 (m, 13H, for one C_6H_5 ring ,two C_6H_4 rings) ; C^{13}-NMR (75.46 MHz, DMSO-*d6*): δ 151.3(C-1),115.2(C-2&6),131.0(C-3&5), 122.1(C-4) of ringA, 93.1(C-4' and C-5'), 176.5(CO-NH attached to Dioxa phospholane ring) of ring B, 162.9(C-2"), 132.5(C-3"), 128.9(C-4"), 125.6(C-5"), 130.8(C-6"), 121.5(C-7"), 148.1(C-8"), 117.4(C-9"), 70.2(N-CH_2-N) of ring C&D and 51.1(C-2&6), 66.5(C-3&5) of ring E. ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.52. Anal. Calcd.(%) for $C_{36}H_{37}N_8O_{10}P$: C55.96, H 4.83, N 14.50; Found: C 55.85, H 4.72, N 14.39.

(4R, 5R) - N'4, N'5-bis-(2- oxo - (4-methyl piperazin-1-ylmethyl) indolin-3-ylidene) - 2-(4-substituted phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide (6g):
Yield: 50%; M.p: 181-183ºC; IR (KBr): 1759 (Indole C = O), 1652 (Carbonyl of CO – NH) and 2918cm^{-1} (CH_2), 1260 cm^{-1} (P=O), 904 cm^{-1}and 1028 cm^{-1} (P-O-$C_{aliphatic}$), 958 cm^{-1}and 1196 cm^{-1} (P-O-$C_{aromtic}$); ^{1}HNMR (300MHz, DMSO-*d6*): δ 2.26 (s, 6H, N–CH_3), 2.46(s, 16H, –CH_2–N–CH_2 of two piperazine rings), 4.03 (s, 4H, –N–CH_2–N–),4.47(s,2H,dioxaphospholane ring) , 8.0 (s, 2H, CO-NH), 7.18-7.86 (m, 13H, for one C_6H_5 ring ,two C_6H_4 rings) ; C^{13}-NMR (75.46 MHz, DMSO-*d6*): δ 150.9(C-1),116.2(C-2&6),131.5(C-3&5), 121.7(C-4) of ringA, 93.5(C-4' and C-5'), 177.3(CO-NH attached to Dioxa phospholane ring) of ring B, 163.0(C-2"), 133.4(C-3"), 128.9(C-4"), 124.2(C-5"), 131.8(C-6"), 122.0(C-7"), 147.8(C-8"), 118.0(C-9"), 70.7(N-CH_2-N) of ring C&D and 50.3(C-2&6), 54.9(C-3&5), 43.1(-N-CH_3) of ring E. ^{31}PNMR (161.89 MHz, DMSO-*d6*): δ -10.64. Anal. Calcd.(%) for $C_{38}H_{43}N_{10}O_8P$: C 57.14, H 5.43, N 17.54; Found: C 57.04, H 5.36, N 17.48.

Biological activity

The antimicrobial activity [28] of chemical compound is influenced by physical and biological characteristics [29]. It has been well established that physiological activity is a function of the chemical structure of compound [30]. Heterocyclic organic compounds containing phosphorous, oxygen, nitrogen or sulfur in the ring system are expected to be more active due to the presence of hetero atoms [31, 32, 33].

In view of this, the synthesized new organophosphorous heterocyclic compounds have been tested for their antimicrobial activity.

Antibacterial activity

The antibacterial activity[34] of (4R, 5R) - N'4, N'5-bis-(2-oxo-1-(piperidin-1-yl methyl)/ (morpholino methyl) / (4-methyl piperazin-1-ylmethyl) indolin-3-ylidene) - 2-(4-substituted phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide **6(a-g)** were screened against the *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) organisms. Most of the compounds exhibited good antibacterial activity against both bacteria. The presence of chlorogroup in the structure has shown increased effect on their antibacterial activity. Penicillin and Streptomycin are tested as reference compounds to compare the activity [35].

Antibacterial activity of (4R, 5R) - N'4, N'5-bis-(2-oxo-1-(piperidin-1-yl methyl)/ (morpholino methyl) / (4-methyl piperazin-1-ylmethyl) indolin-3-ylidene) - 2-(4-substituted phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide 6(a-g)

Compound	R	X	Zone of inhibition (mm)			
			Staphylococcus aureus		*Escherichia coli*	
			250 (µg/disc)	500 (µg/disc)	250 (µg/disc)	500 (µg/disc)
6a	4-H	CH_2	10	12	8	10
6b	4-CH_3	CH_2	9	11	6	9
6c	4-Cl	CH_2	17	18	14	17
6d	4-Br	CH_2	14	16	12	15
6e	4-NO_2	CH_2	16	17	13	16
6f	4-H	O	15	16	11	13
6g	4-H	N-CH_3	12	15	10	12
Penicillin			22	25	20	22
Streptomycin			27	29	25	27

Antifungal activity

The antifungal activity of (4R, 5R) - N'4, N'5-bis-(2-oxo-1-(piperidin-1-yl methyl)/ (morpholino methyl) / (4-methyl piperazin-1-ylmethyl) indolin-3-ylidene) - 2-(4-substituted phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide 6(a-g) were screened against *Aspergillus niger, Helminthosporium oryzae.* Griseofulvin is used as reference compound and exhibited 28 mm and 26 mm inhibition for both fungi at 250 µg / disc [36, 37].

Antifungal activity of (4R, 5R) - N'4, N'5-bis-(2-oxo-1-(piperidin-1-yl methyl)/ (morpholino methyl) / (4-methyl piperazin-1-ylmethyl) indolin-3-ylidene) - 2-(4-substituted phenoxy)-1, 3, 2-dioxa phospholane-4, 5-dicarbohydrazide-2-oxide 6(a-g)

Compound	R	X	Zone of inhibition (mm)			
			Aspergillus niger		*Helminthosporium oryzae*	
			250 (µg/disc)	500 (µg/disc)	250 (µg/disc)	500 (µg/disc)
6a	4-H	CH_2	10	13	9	11
6b	4-CH_3	CH_2	9	12	10	13
6c	4-Cl	CH_2	16	18	15	17
6d	4-Br	CH_2	14	16	12	14
6e	4-NO_2	CH_2	13	15	14	16
6f	4-H	O	11	13	10	12
6g	4-H	N-CH_3	12	15	11	13
Griseofulvin			28	22	26	23

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