Synthesis, anti-microbial properties of 3-(3'-Chloro-4'-nitrophenyl)-2-(substituted phenoxy)-3,4-dihydro-2H-1,3,2 λ^5–benzoxaphosphinin-2-ones

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Abstract:

2-[(3'-chloro-4'-nitro phenylamino) methyl]phenol **1** underwent facile condensation with various phosphorus dichlorides **2a-j** in the presence of TEA in dry THF at 60-65 and afforded corresponding 1,3,2 λ^5 –benzoxaphosphinin-2-one derivatives **3a-j.** Their chemical structures were characterized using IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral studies. All the above compounds were screened for anti-microbial activity and their bioassay showed them to possess significant antimicrobial activity

Key words: Benzoxaphosphin-2-ones, spectral analysis, anti-microbial activity.

Introduction

The chemistry of Organophosphorus heterocyclic compounds received much attention of chemists in past two decades due to their pharmaceutical importance [1] and extensive applications in organic synthesis [2]. They have always attracted considerable interest because of their unique and diverse potential biological properties such as pesticides, antifungal/microbial/lukemic/parasitic/viral/inflammatory/tumor/hypertensive/oxidant and anti human immuno deficiency virus properties [3-5]. Promising anticancer activity of cyclophosphamide [6] and derivatives of cyclophosphamide prompted the studies on six membered compounds as continuation of our work in phosphorus heterocycles [7].

Experimental Section

General

The melting points were determined in open capillary tubes on a Mel-Temp apparatus and were uncorrected. Elemental analyses were performed by Central Drug Research Institute, Lucknow, India. The IR spectra were recorded as KBr pellets on Perkin-Elmer 1000 unit. All ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-300 spectrometer operating at 300 MHz for 1H and 75.46 MHz for ^{13}C. ^{31}P-NMR spectra were recorded on a Varian XL-spectrometer operating at

161.89 MHz. 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). Mass spectral data was recorded on FAB-MS instrument at 70 eV with a direct inlet system.

2-[(3'-chloro-4'-nitro phenylamino) methyl] phenol **1** and various phosphorodichloridates/ phosphorus dichlorides **2a-j** were procured from Sigma-Aldrich Chemical Company, Milwaukee, U.S.A. and were used without further purification.

General procedure for the preparation of 3-(3'-Chloro-4'nitrophenyl)-2-phenoxy -3, 4 dihydro-2H-1, 3, 2 λ^5 –benzoxaphosphinin-2-one (3a-j):

A Solution of respective phosphoro dichloridate **2a** (0.002mmol,0.28ml) in dry THF was added dropwise over a period of 20 minutes to a stirred solution of 2-(3'-chloro-4'-nitrophenyl amino)methyl phenol (**1**) (0.002mmol, 0.278ml) in the presence of TEA (0.003mmol,0.42ml) in 60 ml of dry THF. After completion of addition, the temperature was increased to 60^{o}C-65^{o}C and the stirring was continued for 4-6 h. Progress of the reaction was monitored by TLC analysis using silicagel as adsorbent and ethyl acetate-hexane (1:2) mixture as eluent. Product was isolated from the reaction mixture by separating triethylamine hydrochloride by filtration and evaporation of the filtrate under reduced pressure .The residue was purified by washing with water followed by recrystslization from 2-propanol.

3-(3'-Chloro-4'-nitrophenyl)-2-phenoxy-3,4-dihydro-2H-1,3,2λ^5-benzoxazaphosphinin-2-one ***3a:***

White solid: Yield was found to be 68%, mp 104-106ºC. IR (KBr) cm^{-1}:1267 (P=O), 962(P-O), 1186(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.73-7.89 (m, 12H, Ar-H), 4.22-5.33 (m, 2H, C$\underline{H_2}$). ^{13}C-NMR (DMSO-d_6): δ 43.65 (C-4), 129.20 (C-5), 121.08 (C-6), 127.39 (C-7), 116.03 (C-8), 147.47 (C-9), 129.20 (C-10) 151.98 (C-1'), 116.03 (C-2'), 129.20 (C-3') 138.28 (C-4'), 122.13(C-5'), 109.06(C-6'), 149.70 (C-1"), 116.03 (C-2") 122.13 (C-3"), 138.62 (C-4"), 122.13(C-5") 116.03(C-6"), ^{31}P-NMR (DMSO-d_6): δ -12.89. FAB: m/z (%) 416, 418(M+2). *Anal*. Calcd. for $C_{19}H_{14}ClN_2O_5P$: C, 56.94; H, 3.52; N, 6.99. Found C, 55.88; H, 3.46; N, 6.67

3-(3'-Chloro-4'-nitrophenyl)-2-(4-nitrophenoxy)-3,4-dihydro-2H-1,3,2λ^5-benzoxazaphosphinin-2-one ***3b:***

White solid: Yield was found to be 70%, mp 109-111ºC. IR (KBr) cm^{-1}:1265 (P=O), 937(P-O), 1188(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.68-8.40 (m, 11H, Ar-H), 4.21-5.51 (m, 2H, C$\underline{H_2}$). ^{13}C-NMR (DMSO-d_6): δ 44.84 (C-4), 129.69 (C-5), 121.49 (C-6), 129.12 (C-7), 116.07 (C-8), 145.31 (C-9), 129.12 (C-10) 156.66 (C-1'), 116.07 (C-2'), 129.69 (C-3') 138.85 (C-4'), 124.84(C-5'), 110.33(C-6'), 159.26 (C-1"), 116.50 (C-2") 122.01 (C-3"),144.84 (C-4"), 122.10(C-5") 116.50(C-6"), ^{31}P-NMR (DMSO-d_6):δ -5.09. FAB: m/z (%) 461, 463 (M+2) *Anal*. Calcd. for $C_{19}H_{13}ClN_3O_7P$: C, 49.42; H, 2.84; N, 9.10. Found C, 49.14; H, 2.68; N, 8.94.

3-(3'-Chloro-4'-nitrophenyl)-2-(2-methylphenoxy)-3,4-dihydro-2H-1,3,2λ^5-benzoxazaphosphinin-2-one ***3c:***

White solid: Yield was found to be 71%, mp 72-74ºC. IR (KBr) cm^{-1}:1248 (P=O), 952(P-O), 1180(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.83-7.86 (m, 11H, Ar-H), 4.43-4.58 (m, 2H, C$\underline{H_2}$), 2.14(s,

3H, 2"-CH_3) . ^{31}P-NMR (DMSO-d_6):δ -3.65. *Anal*. Calcd. for $C_{20}H_{16}ClN_2O_5P$: C, 55.76; H, 3.74; N, 6.50. Found . C, 55.28; H, 3.56; N, 6.38

3-(3'-Chloro-4'-nitrophenyl)-2-(3-methylphenoxy)-3,4-dihydro-2H-1,3,2λ^5-benzoxazaphosphinin-2-one ***3d:***

White solid: Yield was found to be 64%, mp 84-86ºC. IR (KBr) cm^{-1}:1246 (P=O), 973(P-O), 1155(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.64-7.84 (m, 11H, Ar-H), 4.41-5.10 (m, 2H, $C\underline{H_2}$), 2.21(s, 3H, 3"-CH_3) . ^{31}P-NMR (DMSO-d_6):δ -4.59. *Anal*. Calcd. for $C_{20}H_{16}ClN_2O_5P$:
C, 55.76; H, 3.74; N, 6.50. Found . C, 55.12; H, 3.66; N, 6.29.

3-(3'-Chloro-4'-nitrophenyl)-2-(4-methylphenoxy)-3,4-dihydro-2H-1,3,2λ^5-benzoxazaphosphinin-2-one ***3e:***

White solid: Yield was found to be 69%, mp 68-70ºC. IR (KBr) cm^{-1}:1243 (P=O), 955(P-O), 1185(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.62-7.91 (m, 11H, Ar-H), 4.34-4.97 (m, 2H, $C\underline{H_2}$), 2.28(s, 3H, 4"-CH_3) . ^{13}C-NMR (DMSO-d_6): δ 41.92 (C-4), 127.81 (C-5), 120.92 (C-6), 128.14 (C-7), 115.62 (C-8), 146.45 (C-9), 128.84 (C-10) 150.84 (C-1'), 113.89 (C-2'), 129.71 (C-3') 137.14 (C-4'), 122.69(C-5'), 111.65(C-6'), 146.87 (C-1"), 114.93 (C-2") 128.68 (C-3"), 131.01 (C-4"), 128.68(C-5") 114.94(C-6"), 23.42(4"-CH_3) ^{31}P-NMR (DMSO-d_6): δ -0.94. FAB: m/z (%) 430, 432 (M+2). *Anal*. Calcd. for $C_{20}H_{16}ClN_2O_5P$: C, 55.76; H, 3.74; N, 6.50. Found . C, 54.82; H, 3.56; N, 6.42.

3-(3'-Chloro-4'-nitrophenyl)-2-(2,4-dimethylphenoxy)-3,4-dihydro-2H-1,3,2λ^5-benzoxazaphosphinin-2-one ***3f:***

White solid: Yield was found to be 73%, mp 130-132ºC. IR (KBr) cm^{-1}:1251 (P=O), 949(P-O), 1192(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.82-7.55 (m, 11H, Ar-H), 4.46-4.89 (m, 2H, $C\underline{H_2}$), 2.16(s, 3H, 2"-CH_3), 2.32(s, 3H, 4"-CH_3) . ^{31}P-NMR (DMSO-d_6): δ -7.50. *Anal*. Calcd. for $C_{21}H_{18}ClN_2O_5P$: C, 56.71; H, 4.08; N, 6.30. Found . C, 56.25; H, 4.02; N, 6.14

3-(3'-Chloro-4'-nitrophenyl)-2-(2-chlorophenoxy)-3,4-dihydro-2H-1,3,2λ^5-benzoxazaphosphinin-2-one ***3g:***

White solid: Yield was found to be 72%, mp 67-69ºC. IR (KBr) cm^{-1}:1252 (P=O), 965(P-O), 1179(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.59-7.93 (m, 11H, Ar-H), 4.25-4.76 (m, 2H, $C\underline{H_2}$). ^{31}P-NMR (DMSO-d_6): δ -6.20. *Anal*. Calcd. for $C_{19}H_{13}Cl_2N_2O_5P$: C, 54.76; H, 3.39; N, 6.72. Found . C, 53.98; H, 3.28; N, 6.56

3-(3'-Chloro-4'-nitrophenyl)-2-(4-chlorophenoxy)-3,4-dihydro-2H-1,3,2λ^5-benzoxazaphosphinin-2-one ***3h:***

White solid: Yield was found to be 66%, mp 75-77ºC. IR (KBr) cm^{-1}:1262 (P=O), 975(P-O), 1183(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.35-7.62 (m, 11H, Ar-H), 4.35-4.86 (m, 2H, $C\underline{H_2}$). ^{31}P-NMR (DMSO-d_6): δ -7.36. *Anal*. Calcd. for $C_{19}H_{13}Cl_2N_2O_5P$: C, 50.58; H, 2.90; N, 6.21. Found . C, 50.26; H, 2.28; N, 6.02

3-(3'-Chloro-4'-nitrophenyl)-2-(2,4-dichlorophenoxy)-3,4-dihydro-2H-1,3,2λ^5-benzoxazaphosphinin-2-one ***3i:***

White solid: Yield was found to be 67%, mp 145-147ºC. IR (KBr) cm^{-1}:1259 (P=O), 960(P-O), 1181(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.33-7.64 (m, 10H, Ar-H), 4.52-4.81 (m, 2H, C$\underline{H_2}$). ^{31}P-NMR (DMSO-d_6): δ -5.12. *Anal*. Calcd. for $C_{19}H_{12}Cl_3N_2O_5P$: C, 46.99; H, 2.49; N, 5.77. Found . C, 46.62; H, 2.38; N, 5.64

Bis-(2-chloro-ethyl)-[3-(3'-chloro-4'-nitrophenyl)-2-oxo-3,4-dihydro-2H-2λ^5-benzo[e][1,3,2]benzoxaxazaphosphinin-2-yl]amine ***3j:***

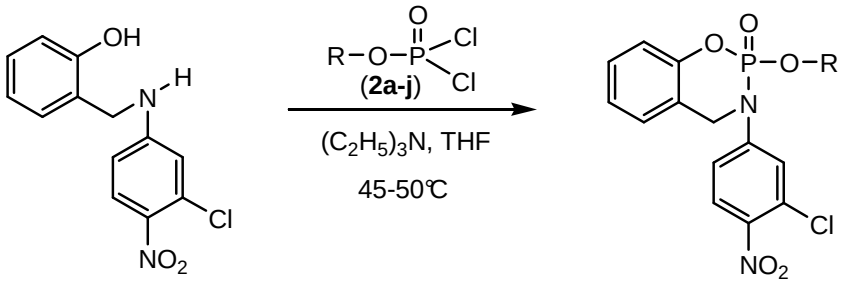
White solid: Yield was found to be 68%, mp 102-104ºC. IR (KBr) cm^{-1}:1249 (P=O), 954(P-O), 1167(O-C). ^{1}H-NMR (DMSO-d_6): δ 6.71-7.92 (m, 7H, Ar-H), 4.54-5.01 (m, 2H, C$\underline{H_2}$), 3.41-3.59(m, 4H, CH_2Cl), 3.16-3.32(m, 4H, NCH_2) . ^{13}C-NMR (DMSO-d_6): δ 44.45 (C-4), 128.19 (C-5), 120.90 (C-6), 128.19 (C-7), 119.47 (C-8), 151.77 (C-9), 129.53 (C-10) 150.41 (C-1'), 109.90 (C-2'), 129.53 (C-3') 138.85 (C-4'), 125.33(C-5'), 109.90(C-6'), 42.59(-N-CH_2), 44.45(-N-CH_2CH_2), ^{31}P-NMR (DMSO-d_6): δ -0.86. FAB mass : m/z (464,M^+) , 466(M+2). *Anal*. Calcd. for $C_{17}H_{17}Cl_3N_3O_4P$: C, 55.76; H, 3.74; N, 6.50. Found . C, 54.82; H, 3.56; N, 6.42.

Results and Discussion

Compounds **3a-j** were synthesized by condensation of phosphonic dichlorides **2a-i** and bis(2-chloroethyl) amine dichloride **2j** with 2-[(3'-chloro-4'-nitro phenylamino)methyl]phenol **1** in the presence of a base in dry THF (Scheme-1)

Characteristic IR absorption were observed in the region 1243-1267cm^{-1} for P=O 952-975 cm^{-1} for P-O and 1155-1192cm^{-1} for C-O stretching frequencies for **3a-j** [8]. The aromatic hydrogens resonate as multiplets at δ 6.33-7.92 in their proton NMR spectra. The CH_2 proton chemical shift appeared as multiplets at δ 4.21-5.51 [9]. The carbon-13 NMR chemical shift for **3a-j** appeared in the expected region. ^{31}P chemical shifts were observed at δ -12.89-(-0.86). FAB mass for few representative compounds for **3a**, **3b**, **3e, 3j** are presented in spectral data [10].

Antibacterial Activity Agar well bioassay was employed for testing antibacterial activity of **3a-j** (Table 1). Diluted inoculum (10^5 CFU/ml) of bacteria was spread on nutrient agar plates. Wells in the agar medium were punched and filled with the title compounds at concentration of 50 and 100mg in each well. The plates were incubated for 24 h at 37 °C for test bacteria. The antimicrobial activity was evaluated by measuring the zone of inhibition against test organisms. Chloramphenicol was used as standard. Controls were maintained with dimethylsulphoxide (DMSO) [11]



(**1**) (**3a-j**)

Synthetic Scheme for 3a-j

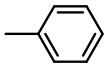
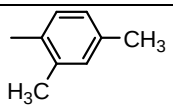
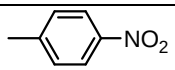
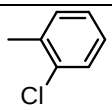
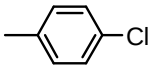
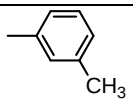
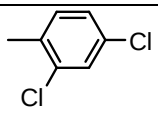
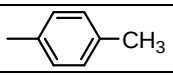
Compd.	R	Compd.	R
2a & 3a		2f & 3f	
2b & 3b		2g & 3g	
2c & 3c		2h & 3h	
2d & 3d		2i & 3i	
2e & 3e		2j & 3j	-N(CH_2CH_2Cl)$_2$

Table 1: Antibacterial activity of 3-(3′-chloro-4′-nitrophenyl)-2-substituted-3,4-dihydro-2*H*-1,3,2λ^{5}benzoxaza-phosphinin-2-one (3a-j)

Compound	Zone of Inhibition (mm)			
	Staphylococcus aureus		*Escherichia coli*	
	250 (µg/disc)	500 (µg/disc)	250 (µg/disc)	500 (µg/disc)
3a	9	10	8	9
3b	8	11	7	8
3c	10	12	8	10
3d	17	18	15	17
3e	6	9	7	9
3f	16	17	14	15
3g	18	19	16	18
3h	14	16	12	14
3i	9	11	7	9
3j	18	19	16	18
Penicillin	22	25	21	22
Streptomycin	27	29	25	27

Table 2: Antifungal activity of 3-(3′-chloro-4′-nitrophenyl)-2-substituted-3,4-dihydro-2*H*-1,3,2λ^5 benzoxaza-phosphinin-2-one (3a-j)

Compound	Zone of Inhibition (mm)			
	Aspergillus niger		*Helminthosporium oryzae*	
	250 (µg/disc)	500 (µg/disc)	250 (µg/disc)	500 (µg/disc)
3a	6	9	5	7
3b	7	10	7	8
3c	8	10	7	9
3d	15	16	13	15
3e	5	8	6	7
3f	14	15	10	12
3g	16	18	14	16
3h	12	14	11	12
3i	7	10	6	7
3j	17	18	14	16
Griseofulvin	28	22	26	23

Minimum inhibitory concentration (MIC) was determined for the compounds **3a-j** (Table 2) that showed total growth inhibition using the protocol described below. The compound concentration of 50m g to 700m g/ml in steps of 25m g/ml was evaluated. Specifically 0.1 ml of standardized inoculum (1-2 X10^7 CFU/ml) was added to each test tube. Two controls (DMSO) with bacteria and antibiotics with bacteria) were maintained for each test sample. The tubes were incubated aerobically at 37 °C for 24 h. [12]

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References

1. J Bergman; HC Vander plas; M Simonyi. Heterocycles in Bio organic Chemistry, RSC, Cambridge, **1991,** 693-694.
2. RY Chen; R Bao. *Synthesis*., **1989**, 618-621.
3. C Fest and KJ Schimidth. The Chemistry of Organophosphorus Pesticides, Springer-Verlog, Berlin, New York, **1982,** 117-118.

4. KG Devine; CMc Guigan; TGO Connor; SR Nicolas; D Kinchington, *AIDS*., **1990**, 4(4), 371-372.
5. PJ Cox. *Biochem Pharmacol*., **1979**, 28, 2045-2049.
6. DL Hill. A Review of Cyclophosphsmide (Charles C Thomas, Springfield Illinois) **1975**
7. A Bala Krishna; MVN Reddy; SK Nayak; M Manjunath; CD Reddy; C Suresh Reddy. *Chem.Pharm.bull*., **2008**, 56(10) 1486-1489
8. RM Silverstein; FX Webster. Spectrometric Identification of Organic Compounds; 6th ed, Chapter-3, John Wiley and Sons, Inc: New York, U.S.A., **1998**, p.104-105
9. MSR Naidu ; CN Raju. *Indian J.Chem*., **1988**, 27B, 88-90.
10. MM Crutchfield; CH Dungan; JH Letcher; U Mark; JR Van Wazer. ^{31}P Nuclear Magnetic Resonance. Inter Science Publishers: New York, U.S.A, **1967**; p 155-157.
11. DV Mangte; SP Deshmukh; DD Bhokare; A Arti Deshpande. *Indian J Pharm*., **2007**, 69, 295-298.
12. E Omer. *Biologia*., **2006**, 61, 275-278.