Synthesis and antibacterial activity of new 2-aryloxy-6-bromo-3-(4-chlorophenyl)-3,4-dihydrobenzo[*e*][1,3,2]oxazaphosphinine 2-oxides

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Abstract

2-Aryloxy-6-bromo-3-(4-chlorophenyl)-3,4-dihydro-benzo[*e*][1,3,2]oxazaphosphinine 2-oxides **3** have been synthesized by the reaction of 4-bromo-2-[(4-chlorophenylamino)methyl]phenol (**1**) with various aryl phosphorodichloridates **2a-i** in the presence of triethylamine in dry toluene-tetrahydrofuran at 45–50 °C. Products **3** were characterized by IR, ^{1}H, ^{13}C, and ^{31}P NMR spectra, and their antibacterial activity was evaluated.

Keywords: Benzoxazaphosphinine 2-oxides, antibacterial

Introduction

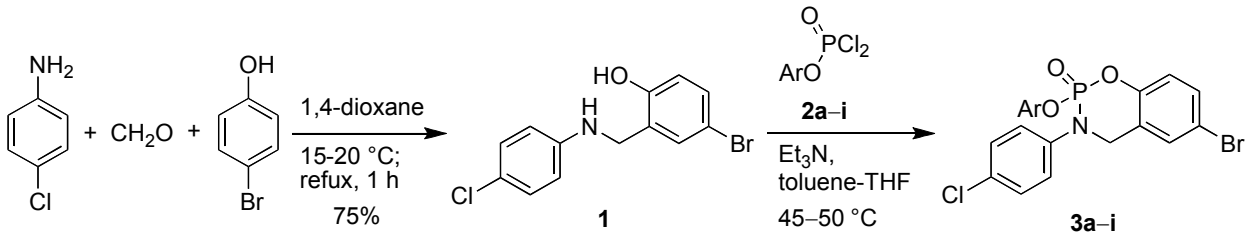
Organophosphorus compounds being ubiquitous in nature have found multifaceted applications. 1,3,2-Oxazaphosphinine derivatives, cyclophosphamide and its analogues, isophosphamides are clinically useful anticancer drugs;[1–2] organophosphorus esters are used as pesticides[3] and insecticides.[4a–b] In view of this, a series of 2-aryloxy-6-bromo-3-(4-chlorophenyl)-3,4-dihydro-benzo[*e*][1,3,2]oxazaphosphinine 2-oxides **3** has been synthesized.

Results and Discussion

The synthetic route involves preparation of 4-bromo-2-[(4-chlorophenylamino)methyl]phenol (**1**) (by condensation of 4-chloroaniline, 4-bromophenol and aqueous formaldehyde[10]) followed by the cyclocondensation of **1** with aryl phosphorodichloridates[5] **2a-i** in the presence of triethyl-amine in toluene-tetrahydrofuran at 45–50 °C (Scheme 1). The formation of 2-aryloxy-6-bromo-3-(4-chlorophenyl)-3,4-dihydrobenzo[*e*][1,3,2]oxazaphosphinine 2-oxides **3a-i** involves a nucleophilic attack of the amino and hydroxy groups of **1** at the electrophilic phosphorus of

dichloridate **2**. The reactions were completed within 4–6 hours. Product structures **3** were characterized by elemental analyses, IR, ^{1}H, ^{13}C, and ^{31}P NMR spectra (Tables 1 and 2). Compounds **3a**–**i** exhibit P=O stretching frequencies in the region 1246–1270 cm^{-1}.

Characteristic absorption bands for P–O and O–$C_{aromatic}$ stretching vibrations are observed in the region 948–992 and 1148–1190 cm^{-1}, respectively.[6–8] The ^{1}H NMR spectra of **3a**–**i** exhibit a complex multiplet at δ 6.35–8.26 for the aromatic protons. The ^{13}C NMR chemical shifts of the aromatic and heterocyclic rings **3a**–**d**,**f**,**i** were observed in the expected ranges.[9a,b]



2, 3	Ar	2, 3	Ar
a	C_6H_5	**f**	2-ClC_6H_4
b	2-MeC_6H_4	**g**	2,4-$Cl_2C_6H_3$
c	4-$O_2NC_6H_4$	**h**	4-BrC_6H_4
d	3-MeC_6H_4	**i**	2,5-$Me_2C_6H_3$
e	4-ClC_6H_4		

Scheme 1

Antibacterial activity

For the bioassay, the compounds were dissolved in DMSO. No antibacterial activity was noted in the solvent employed. Ciprofloxacin (Hi-media) controls were included for comparison with compounds **3a**–**i**. All samples were tested in triplicate, and average results are reported.

The compounds were assayed for antibacterial activity against six registered bacterial isolates, which were obtained from the NCIM (National Collection of Industrial Microorganisms, National Chemical Laboratories, Pune-411 003, India): Two Gram positive bacterial isolates, *Staphylococcus aureus* (NCIM No. 5021, ATCC No. 25923), *Bacillus subtilis* (NCIM No. 2063, ATCC No. 6633) and four Gram negative bacteria, *Escherichia coli* (NCIM No: 2931, ATCC No. 25922), *Pseudomonas aeruginosa* (NCIM No. 5029, ATCC No. 27853), *Salmonella typhimurium* (NCIM No. 2501, ATCC No, 23564) and *Klebsiella pneumoniae* (NCIM No. 2957). The bacteria were grown on (Hi-media) nutrient agar and sub cultured as needed.

Conclusions

A simple synthesis of 2-aryloxy-6-bromo-3-(4-chlorophenyl)-3,4-dihydrobenzo[*e*][1,3,2]oxaza-phosphinine 2-oxides **3** is presented. The reactions were performed in dry toluene-tetrahydrofuran in the presence of triethylamine, because all reactants are readily soluble in this solvent at room temperature, and products **3** do not decompose at the reaction temperature of 45-50 °C.

The new phosphorus heterocycles **3** may be potential insecticides and antibacterials. The results of the antibacterial study (Table 3) shows that all tested compounds **3a**–**i** inhibit the growth of selected bacteria at 40 μg/mL. **3d, 3e, 3f** and **3g** inhibit the growth of both Gram positive and Gram negative bacteria at lower concentration (20 μg/mL). The highest activity was obtained by **3c** and **3d**, but **3c** shows more potential activity when compared with **3d** except in the case of *Staphylococcus aureaus*. **3b** does not inhibit the growth of Gram positive bacteria at 40 μg/mL. **3d** shows moderate inhibition of Gram negative bacteria at 20 μg/mL.

Experimental Section

General Procedures. Melting points were determined on Mel-Temp apparatus. Elemental analyses were performed by the Central Drug Research Institute, Lucknow, India. The IR spectra (KBr pellets) were recorded on a Perkin-Elmer 1430 unit. ^{1}H, ^{13}C, and ^{31}P NMR spectra ($CDCl_3$ solutions) were recorded on a AMX-400 MHz spectrometer operating at 400, 100, and 161.9 MHz, respectively; Chemical shifts were referenced to TMS (^{1}H and ^{13}C) and 85% H_3PO_4 (^{31}P).

4-Bromo-2-[(4-chlorophenylamino) methyl]phenol (1). To a stirred and cooled (10–20 °C) solution of aqueous formaldehyde (37%; 7.5 mL, 0.1 mol) in 1,4-dioxane (30 mL) was added 4-chloroaniline (11.10 g, 0.1 mol) at 15–20°C. To the stirred reaction mixture a solution of 4-bromophenol (17.3 g, 0.1 mol) in 1,4-dioxane (20 mL) was added dropwise through a dropping funnel; the resulting solution was heated at gentle reflux for 1 h. Upon removal of 1,4-dioxane and water by a rotary evaporator a sticky mass remained. Recrystallization of the residue with 95% ethanol yielded **1** (26.5 g, 75%); mp 80–82 °C. IR (KBr): $\tilde{\nu}$ 3251 (N-H), 3436 (br, O-H) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.34 (s, 2H, CH_2), 4.77 (s, 1H, NH), 8.23 (br s, 1H, OH), 6.75–7.32 (m, 7H, H_{Ar}). Anal. Calcd for $C_{13}H_{11}BrClNO$ (312.59): C, 49.95; H, 3.55; N, 4.48. Found: C, 49.89; H, 3.51; N, 4.51.

6-Bromo-3-(4-chlorophenyl)-2-(2-methylphenyloxy)-3,4-dihydrobenzo[*e*][1,3,2]oxazaphos-phinine 2-oxide (3b). A solution of 2-methylphenyl phosphorodichloridate (**2b**, 0.42 g, 0.002 mol) in dry toluene (25 mL) was added dropwise over a period of 20 min to a stirred solution of 4-bromo-2-[(4-chlorophenylamino)methyl]phenol (**1**, 0.62 g, 0.002 mol) and triethylamine (0.40 g, 0.004 mol) in dry toluene (30 mL) and tetrahydrofuran (10 mL) at room temperature. The reaction mixture was stirred for 2 h before the temperature was raised to 45–50°C, and stirring was continued for an additional 4 h. After completion of the reaction (monitored by TLC)

triethylamine hydrochloride was filtered off and the solvent was removed under reduced pressure. The resulting residue was washed with water and recrystallized from aqueous 2-propanol to afford pure **3b** (0.27 g, 57%); mp 67–69 °C.

Compounds **3a**,**c**–**i** were prepared by the same procedure; analytical and spectral data of products **3a**–**i** are listed in Tables 1 and 2.

Disc diffusion bioassay. A suspension of approximately 1.5×10^8 bacterial cells per mL was used. Sterile normal saline was prepared as described by Forbes *et al.*[11] and 1.5 mL of it was uniformly spread on Nutrient Agar (Hi-media) in 12 × 1.2 cm glass Petri dishes, left aside for 15 min and excess of the suspension was drained and discarded properly. For the agar disc diffusion method, the test compound was introduced to the disc and then allowed to dry. Thus, the disc was completely saturated with the test compound. Then the disc was introduced to the upper layer of the medium with the bacteria. The Petri dishes were incubated at 37 °C for 24 h. Bioactivity was determined by measuring the Diameter of Inhibition Zones (DIZ) [mm]. Compound concentrations of 20 and 40 μg/mL were evaluated. Each test was done in triplicate, and the mean of the diameter of the inhibition zones was calculated. No antibacterial activity was noted for the solvent employed in the test.[12] (Table 3).

Table 1. Physical and IR spectral data of **3a**–**i**

3	mp [°C]	Yield (%)	Mol. Formula (mol. wt.)	Elemental analysis % Found (Calcd)			IR [cm^{-1}]		
				C	H	N	P=O	P-O	O-C
a	83–84	68	$C_{19}H_{14}BrClNO_3P$ (450.66)	50.68 (50.64)	3.07 (3.13)	3.05 (3.11)	1252	962	1171
b	67–69	57	$C_{20}H_{16}BrClNO_3P$ (464.69)	51.76 (51.70)	3.38 (3.47)	2.97 (3.01)	1239	965	1173
c	110–111	62	$C_{19}H_{13}BrClN_2O_5P$ (495.66)	46.05 (46.04)	2.52 (2.64)	5.72 (5.65)	1270	966	1190
d	69–71	61	$C_{20}H_{16}BrClNO_3P$ (464.69)	51.87 (51.70)	3.43 (3.47)	3.06 (3.01)	1252	968	1179
e	79–81	64	$C_{19}H_{13}BrCl_2NO_3P$ (485.10)	47.24 (47.04)	2.71 (2.70)	2.92 (2.89)	1252	985	1152
f	68–70	60	$C_{19}H_{13}BrCl_2NO_3P$ (485.10)	47.22 (47.04)	2.64 (2.70)	2.86 (2.89)	1248	982	1148
g	77-79	62	$C_{19}H_{12}BrCl_3NO_3P$ (519.55)	43.06 (43.92)	2.28 (2.33)	2.68 (2.70)	1261	992	1136
h	86-88	61	$C_{19}H_{13}Br_2ClNO_3P$ (529.56)	43.21 (43.10)	2.42 (2.47)	2.61 (2.64)	1246	960	1182
i	112-114	58	$C_{21}H_{18}BrClNO_3P$ (478.71)	52.79 (52.69)	3.74 (3.79)	2.89 (2.93)	1262	948	1179

Table 2. ^{1}H, ^{13}C, and ^{31}P NMR spectral data (δ, $CDCl_3$) of **3a–i**

3	^{1}H NMR	^{13}C NMR	^{31}P NMR
a	6.99–7.45 (m, 12H, H_{Ar}) 4.50–4.95 (m, 2H, CH_2)	46.76, 132.31, 119.27, 135.12, 115.48, 149.70, 111.89, 156.12, 115.55, 124.63, 124.29, 144.19, 114.94, 127.11, 126.30	–9.23
b	6.97–7.45 (m, 11H, H_{Ar}) 4.57–4.95 (m, 2H, CH_2) 2.10 (s, 3H, CH_3)	16.08, 52.04, 131.57, 132.39, 120.80, 148.97, 119.89, 149.50, 124.71, 129.21, 125.40, 127.22, 120.72, 140.43, 117.13, 129.57, 125.60	–7.32
c	6.97–8.26 (m, 11H, H_{Ar}) 4.55–5.00 (m, 2H, CH_2)	52.19, 117.65, 132.66, 120.42, 162.12, 116.22, 125.76, 142.30, 145.30, 117.09, 129.83, 125.83	–9.54
d	6.89–7.45 (m, 11H, H_{Ar}) 4.49–4.95 (m, 2H, CH_2) 2.30 (s, 3H, CH_3)	21.31, 51.98, 131.48, 132.35, 120.83, 149.57, 150.23, 117.10, 140.24, 125.40, 129.540, 140.51, 126.34, 124.63	–7.36
e	6.35–7.50 (m, 11H, H_{Ar}) 4.48–4.97 (m, 2H, CH_2)	----	–8.87
f	6.91–7.56 (m, 11H, H_{Ar}) 4.50–5.08 (m, 2H, CH_2)	52.18, 130.14, 117.24, 130.87, 155.56, 120.53, 148.45, 123.51, 128.04, 124.60, 126.74, 146.46, 116.84, 129.60, 125.40	–7.30
g	6.89–7.92 (m, 10H, H_{Ar}) 4.22–4.96 (m, 2H, CH_2)	---	–8.25
h	6.35–7.45 (m, 11H, H_{Ar}) 4.48–4.88 (m, 2H, CH_2)	---	–8.84
i	6.73–7.44 (m, 10H, H_{Ar}) 4.49–4.95 (m, 2H, CH_2) 2.24 (s, 6H, 2 CH_3)	21.22, 51.93, 131.40, 117.00, 132.31, 120.75, 149.60, 150.06, 124.62, 129.58, 120.83, 139.79, 140.55, 117.50, 129.48, 125.35	–7.36

Table 3. Antibacterial activity of compounds **3a–i** in terms of diameter of inhibition zones (DIZ) n/a indicates no activity

	Staphylococcus aureus		*Bacillus subtilis*		*Escherichia coli*		*Salmonella typhi*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*	
μg/mL	20	40	20	40	20	40	20	40	20	40	20	40
3	DIZ [mm]											
a	10	14	10	13	n/a	10	n/a	10	n/a	11	n/a	11
b	10	13	11	15	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
c	10	n/a	12	15	11	13	12	13	12	14	11	14
d	10	15	11	14	10	12	10	12	12	14	11	13
e	10	13	10	13	10	12	10	13	10	13	11	14
f	10	12	11	14	11	14	10	12	11	13	10	12
g	11	13	11	14	10	12	10	12	11	14	10	11
h	10	12	n/a	12	n/a	10	n/a	10	10	12	n/a	12
i	n/a	10	12	14	n/a	10	n/a	11	10	12	n/a	10
Cifro-floxacin	22		24		30		25		28		25	

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