Synthesis and Antimicrobial activity of 2, 10 - dibromo dibenzo [*d,g*] [1,3,6,2] dioxathiaphosphocin 6-sulfido-6-amino acid esters

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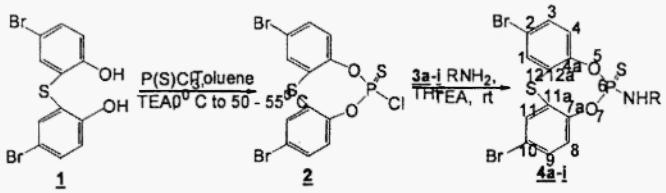
Abstract : Synthesis of 2,10 - dibromo dibenzo [*d,g*][1,3,6,2] dioxathiaphosphocin 6-sulfido-6-amino acid esters **4a-i** were accomplished through a two step process. This involved prior preparation of the monochloride **2** as 2,10-dibromo-6-chloro dibenzo [*d,g*][1,3,6,2] dioxa- thiaphosphocin 6-sulfide and its subsequent reaction with the ester of amino acids **3a-i** in dry tetrahydrofuran in the presence of triethylamine at various temperatures. They were characterized by elemental analysis, IR, ^{1}H, ^{13}C and ^{31}P spectral data.

Introduction

Phosphoramides substituted with an amino acid ester are an important class of rationally designed therapeutics especially with antineoplastic properties (1-5). The attachment of an amino acid group to the phosphate moiety is expected to increase their cellular uptake and thus enhance their chemotherapeutic properties (3,4). Recently, McGuigan reported that phosphorus triester derivatives of 3-azido-3-deoxythymidine (AZT) bearing amino acid moieties did have anti - HIV activity (6). Further, hydrolysis of these novel heterocycles may release products of limited toxicity in the host system (7). In view of this back ground the title compounds were designed incorporating active pharmacophoric structural features, synthesized them and screened for their antimicrobial activity.

Result and discussion

The synthetic route (Scheme 1) involves the cyclization of 5, 5' -dibromo -2, 2' - dihydroxy diphenyl sulfide **1** with thiophosphoryl chloride in dry toluene in the presence of triethylamine (TEA) obtained the intermediate monochloride **2**, 2,10-dibromo 6-chloro dibenzo [*d,g*][1,3,6,2] dioxathiaphosphocin 6-sulfide. It was reacted with amino acid methyl and ethyl ester hydrochlorides **3a-i** in dry tetrahydrofuran(THF) in the presence of triethylamine to get title compounds **4a-i** in good yields.



Compd.	R	Compd.	R
4a	$-CH_2-COOCH_3$	4f	$-CH(CH_3)-CH(CH_3)-COOCH_3$
4b	$-CH_2-COOC_2H_5$		CH_2CH_3
4c	$-CH(C_6H_5)-COOCH_3$	4g	$-CH(CH_2CH_2SCH_3)-COOCH_3$
4d	$-CH(CH_3)-COOCH_3$	4h	$-CH(CH_2CH_3)-COOCH_3$
4e	$-CH(CH_2CH(CH_3)_2)-COOCH_3$	4i	CH_3OOC-C$_6$H$_4$-

Scheme 1

The second step of the reaction was completed at room temperature with stirring for 6 -7 hours for **4a-h**. The same reaction was completed at 40-45^0 C for **4i** in 7 hours. Progress of the reaction is monitored by thin layer chromatography (TLC) analysis of the reaction mixture. The title compounds **4a-i** were purified by flash chromatographic method using hexane – ethyl acetate step gradient mixtures as an eluent. Their structures were established by elemental analyses, IR, ^{1}H, ^{13}C and ^{31}P NMR data (Tables 1-3).

IR data (Table 1) of members of **4** showed bands (8) at 774-798 cm^{-1} (P=S), 1725-1746 cm^{-1} (C=O) and 3402-3434 cm^{-1} (P-NH).

In the ^{1}H NMR spectra (Table 2), aromatic protons resonated at δ 6.91-7.90. The singlet at δ 10.20-11.70 is assigned to P-NH. The ^{13}C NMR chemical shifts (Table 3) were interpreted based on comparison with carbon chemical shifts of **3** and related system (9, 10). Oxygen bearing carbons of the title compounds gave doublets in the region 153.2-153.5 ppm (J=8.3-9.2 Hz). ^{31}P NMR spectra of **4a**, **4b** and **4i** showed only one phosphorus resonance signal (11, 12) in the range of 48.14-48.25 ppm. But compounds **4c-h** (Table 1) gave two ^{31}P resonance signals in the region of 48.31-52.01 ppm to 56.84-63.51 ppm and this lends to the supposition that they may exist in two conformers (13) with sufficient internal energy difference and considerable stability to enables measurement of their ^{31}P NMR chemical shifts.

Antimicrobial Activity

The compounds **4a-i** (Table 4) were screened for their antifungal activity against *Aspergillus niger* and *Helminthosporium* species along with the standard fungicide Bavestein.

Table 1: Physical, IR and ^{31}P NMR spectral data of 4

Compd	mp (^{0}C)	Yield (%)	Molecular formula	Elemental analysis Found(Calcd)%			IR(cm^{-1})[a]			^{31}P NMR[b,c]
				C	H	N	P=S	C=O	P-NH	
4a	154-155	68	$C_{15}H_{12}Br_2O_4PNS_2$	34.31 (34.27	2.30 2.28	2.67 2.60)	774	1746	3434	48.21
4b	102-103	62	$C_{16}H_{14}Br_2O_4PNS_2$	35.64 (35.59	2.62 2.68	2.58 2.52)	776	1745	3431	48.25
4c	141-142	70	$C_{21}H_{16}Br_2O_4PNS_2$	41.96 (41.89	2.68 2.67	2.33 2.40)	775	1732	3422	48.93,58.415
4d	150-151	66	$C_{16}H_{14}Br_2O_4PNS_2$	35.64 (35.59	2.62 2.70	2.58 2.64)	776	1746	3402	48.51,62.49
4e	158-160	67	$C_{19}H_{20}Br_2O_4PNS_2$	39.26 (39.18	3.47 3.39	2.41 2.50)	775	1746	3419	52.01,58.43
4f	177-179	62	$C_{19}H_{20}Br_2O_4PNS_2$	39.26 (39.19	3.47 3.44	2.41 2.49)	774	1725	3420	51.26, 58.44
4g	123-124	69	$C_{18}H_{18}Br_2O_4PNS_3$	36.08 (36.00	3.03 3.12	2.34 2.40)	798	1730	3427	48.31, 63.51
4h	160-162	66	$C_{17}H_{16}Br_2O_4PNS_2$	36.91 (36.84	2.92 2.86	2.53 2.60)	774	1730	3431	50.98, 56.84
4i	190-192	72	$C_{20}H_{14}Br_2O_4PNS_2$	40.91 (40.87	2.40 2.48	2.38 2.39)	774	1732	3419	48.14

[a]Recorded as KBr pellets
[b]Recorded in $CDCl_3$
[c]Chemical shifts in ppm from 85% phosphoric acid

Table 2: ^{1}H NMR Spectral data[a,b] of 4

Compd.	Ar-H	N-H	Amino acid ester-H
4a	6.99-7.75 (m,6H)	11.70 (s, 1H)	3.15 (s, 3H, OCH_3) 4.01 (s, 2H, CH_2)
4b	6.98-7.74 (m, 6H)	11.64 (s, 1H)	3.65 (s, OCH_2), 3.46 (s, 2H, CH_2), 3.14(s, 3H, CH_3)
4c	7.00-7.82(m, 11H)	11.48 (s, 1H)	3.17(s, 3H, OCH_3), 4.10(s, 1H, CH)
4d	6.97-7.75 (m, 6H)	11.60 (s, 1H)	3.19(s, 3H, OCH_3), 3.65(m, 1H, CH), 1.41 (d, 3H, $CH\text{-}CH_3$)
4e	7.00-7.84 (m, 6H)	10.20 (s, 1H)	3.26(s, 3H, OCH_3), 4.46-4.52(m,1H, -CH-CH_2-CH-$(CH_3)_2$),1.42-1.55(m, 2H, -CH-CH_2-CH-$(CH_3)_2$ 1.22-1.31(m, 1H, CH-CH_2-CH-$(CH_3)_2$),1.10(d,6H, J=12.7 Hz,-CH-CH_2-CH-$(CH_3)_2$)
4f	7.00-7.83 (m, 6H)	11.10 (s, 1H)	3.60(s, 3H, OCH_3), 4.07-4.11(m, 1H, -CH-CH(CH_3)-CH_2-CH_3), 3.07-3.19(m,1H,-CH-CH(CH_3)-CH_2-CH_3), 1.17(d, J=12.1 Hz,3H, -CH-CH(CH_3)-CH_2-CH_3) ,2.17-2.35(m, 2H, -CH-CH(CH_3)-CH_2-CH_3), 0.97(t, 3H, -CH-CH(CH_3)-CH_2-CH_3)
4g	6.91-7.81 (m, 6H)	11.50 (s, 1H)	3.90(s, 3H, OCH_3), 4.29-4.45(m, 1H, -CH-CH_2-CH_2-S-CH_3),1.38-1.40(m, 2H, -CH-CH_2-CH_2-S-CH_3), 3.11-3.17(m,2H, -CH-CH_2-CH_2-S-CH_3),4.09(s,3H, -CH-CH_2-CH_2-S-CH_3)
4h	7.01-7.70 (m, 6H)	10.80 (s, 1H)	3.23(s, 3H, OCH_3), 4.50 (m, 1H, NH-CH-), 2.16(m, 2H, -CH_2-CH_3), 1.43(t,3H,-CH_2-CH_3)
4i	6.98-7.90(m,10H)	11.68 (s, 1H)	3.14 (s, 3H, OCH_3)

[a]Chemical shifts in ppm and J(Hz) given in parenthesis
[b]Recorded in $CDCl_3$

Table 3: ^{13}C NMR Spectral data[a] of some members of 4

Compd	Chemical shifts (in ppm)
4a	138.4 (s, 2C,C-1&11), 133.8 (s, 2C,C-2&10), 129.8 (s, 2C, C-3&9), 117.0(s, 2C, C-4&8),153.5 (d,*J*=8.9 Hz, 2C, C-4a&7a), 126.6 (s, 2C, C-11a &12a),47.2 ($\underline{C}H_2$-COOCH$_3$), 173.1 (CO-OCH$_3$), 57.1 (O$\underline{C}$H$_3$).
4b	138.5 (s, 2C,C-1&11), 133.8 (s, 2C,C-2&10), 129.8 (s, 2C, C-3&9), 117.0 (s, 2C, C-4&8),153.4 (d,*J*=9.2 Hz,2C, C-4a&7a), 126.6 (s, 2C, C-11a &12a),43.3 ($\underline{C}H_2$-COOC$_2$H$_5$), 172.1 ($\underline{C}$O-OC$_2$H$_5$), 57.1 (O$\underline{C}$H$_2$), 13.3(OCH$_2$-$\underline{C}$H$_3$).
4d	138.4 (s, 2C,C-1&11), 133.2 (s, 2C,C-2&10), 129.7 (s, 2C, C-3&9), 117.3(s, 2C, C-4&8),153.2 (d,*J*=8.3 Hz, 2C, C-4a&7a), 127.1 (s, 2C, C-11a &12a),47.4 (CH (CH$_3$)), 23.6 (CH$_3$), 171.9 (CO-OCH$_3$), 56.3 (OCH$_3$).
4e	139.0 (s, 2C,C-1&11), 134.5 (s, 2C,C-2&10), 129.1 (s, 2C, C-3&9), 118.0 (s, 2C, C-4&8),153.2 (d,*J*=8.6 Hz, 2C, C-4a&7a), 125.9 (s, 2C, C-11a &12a),43.0 (-$\underline{C}$H-COO CH$_3$), 34.4 (-$\underline{C}$H$_2$-CH-(CH$_3$)$_2$), 26.0 (-CH$_2$-$\underline{C}$H (CH$_3$)$_2$, 24.7, 24.9 (-CH$_2$-CH ($\underline{C}$H$_3$)$_2$), 173.4 ($\underline{C}$OOCH$_3$), 57.3 (OCH$_3$).
4h	138.5 (s, 2C,C-1&11), 133.8(s, 2C,C-2&10), 131.3 (s, 2C, C-3&9), 122.5 (s, 2C, C-4&8),153.2 (d,*J*=8.5 Hz, 2C, C-4a&7a), 129.2 (s, 2C, C-11a &12a),44.2 (-$\underline{C}$H-COOCH$_3$), 30.1($\underline{C}$H$_2$-CH$_3$), 25.1(CH$_2$-$\underline{C}$H$_3$), 172.8($\underline{C}$OOCH$_3$),57.6(OCH$_3$).
4i	138.4 (s, 2C, C-1&11), 133.8, (s, 2C,C-2&10), 129.8 (s, 2C, C-3&9), 116.9 (s, 2C, C-4&8),153.4 (d,*J*=8.6 Hz, 2C, C-4a&7a), 126.6(s, 2C, C-11&12a),172.6 ($\underline{C}$OOCH$_3$), 57.2 (OCH$_3$).

[a] *J* (Hz) given in parenthesis

Table 4: Antifungal and Antibacterial Activities of Compounds 4 in Terms of Zone of Inhibition (mm)

Compd.	Fungi						Bacteria					
	Aspergillus niger			Helminthosporium oryzae			Escherichia coli			Staphylococcus aureus		
	100	50	25	100	50	25	100	50	25	100	50	25
4a	-	-	-	11	6	-	14	8	4	11	8	6
4b	11	8	4	14	9	4	13	8	4	10	8	6
4c	13	9	6	15	10	6	15	12	8	12	9	5
4d	12	10	8	16	9	4	15	12	7	18	14	9
4e	9	5	3	14	11	9	-	-	-	10	8	-
4f	9	5	-	9	-	-	10	5	-	-	-	-
4g	14	10	9	13	12	8	12	8	-	11	8	6
4h	13	9	8	12	10	6	8	5	-	-	-	-
4i	13	9	9	11	9	6	8	4	-	12	10	6
Control	8	5	-	12	9	-	10	6	-	9	5	-

Concentrations expressed in ppm

'-' indicates no activity

Disc diffusion method [14] was followed for screening the compounds at three different concentrations (25, 50, 100 ppm). Their antibacterial activity was also evaluated according to the disc diffusion method [15, 16] at three different concentrations against *Escherichia coli* and *Staphylococcus aureus* by comparing with standards Streptomycin and Penicillin.

It is interesting to observe that all compounds **4a-i** exhibited higher anti-fungal and anti- bacterial activity when compared with that of standards. The highlight is that all the compounds exhibited very high activity against fungi and some compounds were twice more effective than the standard *Bavestein*. Similarly it is gratifying to observe that these compounds are extremely more effective against bacteria *aureus*.

Experimental

All melting points were determined on a Mel-Temp apparatus and were uncorrected. Elemental analyses were performed by the Central Drug Research Institute, Lucknow, India. The IR spectra were recorded as KBr pellets on a Perkin - Elmer 1000 unit.The ^{1}H, ^{13}C and ^{31}P spectra were taken on AMX 400 MHz NMR spectrometer operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C and 161.9 MHz for ^{31}P NMR. Compounds were dissolved in $CDCl_3$ and chemical shifts were referenced to TMS (^{1}H and ^{13}C NMR) and 85% H_3PO_4 (^{31}P NMR).

5, 5' -Dibromo -2, 2' - dihydroxy diphenyl sulfide **1** and the amino acid ester hydrochlorides (17) **3a-i** were prepared according to the reported procedures.

2, 10-dibromo 6-chloro dibenzo [*d,g*][1,3,6,2] dioxathiaphosphocin 6-sulfide 2

General procedure

A solution of thiophosphoryl chloride (0.85 g, 5 mmol) in 25mL of dry toluene was added drop wise over a period of 20 minutes to a stirred solution of (1.88 g, 5 mmol) 5,5' - dibromo -2,2' - dihydroxy diphenyl sulfide **1** and (1.01 g, 10 mmol) triethylamine in 50 mL of toluene at 0-5^{0} C. After the addition, the reaction mixture was stirred at room temperature for two hours, and then stirred at 50-55^{0} C for another four hours. Completion of the reaction is monitored by TLC analysis. The reaction mixture was filtered and the solvent from the filtrate was evaporated under reduced pressure. The residue was washed with petroleum ether (60-80^{0} C) and used for the second step reactions without further purification.

2, 10-dibromo dibenzo [*d, g*] [1, 3, 6, 2] dioxathiaphosphocin 6-sulfido-6-anthranilic acid methyl ester 4i

A mixture of anthranilic acid methyl ester hydrochloride (**3i** ,0.37 g, 2 mmol), 2,10-dibromo-6-chloro dibenzo [*d,g*][1,3,6,2] dioxathiaphosphocin 6-sulfide (**2**, 0.94 g, 2 mmol) and triethylamine (0.41 g, 4 mmol) in 50 mL of dry THF was stirred at 40-45^{0} C for 7 hours. The progress of the reaction was monitored by TLC analysis. Triethylamine hydrochloride was separated by filtration and the solvent was evaporated under reduced pressure. Purification of the residue was achieved by flash chromatography on silica gel, using hexane-ethyl acetate (8:2) as an eluent, yield 1.13 g (72%), mp 190-192^{0}C.

Compounds **4a-h** were prepared by adopting the same procedure.

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