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Synthesis and Antimicrobial Activity of 5,5′-Dimethyl-2-Oxido-[1,3,2]-Dioxaphosphorinane-2-yl-Amino Carboxylates

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ABSTRACT: *Synthesis of several 5,5-dimethyl-2-oxido-[1,3,2]-dioxaphosphorinane-2-yl-amino carboxylates (**4a–j**) was accomplished through a two-step process. This involves prior preparation of the intermediate monochloride (**2**), 2-chloro-5,5-dimethyl [1,3,2]dioxaphosphorinane-2-oxide and its subsequent reaction with various amino acid esters (**3a–j**) in dry tetrahydrofuran in the presence of triethyl amine at room temperature. They were characterized by elemental analysis, IR, ^{1}H, ^{13}C, ^{31}P NMR, and mass spectral data. Their antifungal and antibacterial activity is also evaluated. Majority of these compounds exhibited moderate antimicrobial activity in the assay.* © 2008 Wiley Periodicals, Inc. Heteroatom Chem 19:256–260, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20426

INTRODUCTION

Phosphoramides substituted with an amino acid ester are an important class of rationally designed therapeutics especially with antineoplastic [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 and Narasimhan reported that phosphorus triester derivatives of 3-azido-3-deoxythymidine (AZT) bearing amino acid moieties exhibited enhanced anti-HIV activity [6]. 5′-Phosphorylated AZT with tryptophan ethyl ester attached at phosphorus exhibited 8-fold increase in anti-HIV activity compared to free AZT without any symptoms of toxicity [7]. Furthermore, hydrolysis of these novel heterocycles may release products of limited toxicity in the host system [8]. In view of this background, the title compounds were designed incorporating active pharmacophoric structural features, synthesized, characterized, and screened for their antimicrobial activity.

RESULTS AND DISCUSSION

The synthetic route (Scheme 1) involves the cyclization of 2,2-dimethyl-1,3-propanediol (**1**) with phosphorusoxychloride in dry toluene in the presence of triethylamine (TEA), and we obtained the intermediate monochloride (**2**). It was reacted with amino acid methyl/ethyl ester hydrochlorides (**3a–j**) in dry tetrahydrofuran (THF) in the presence of TEA to get title compounds **4a–j** in good yields.

The second step of the reaction was completed at room temperature with stirring for 5–6 h to get **4a–j**. Progress of the reaction was monitored by thin layer chromatography (TLC) analysis of the reaction mixture. The title compounds **4a–j** were purified by flash chromatographic method using ethylacetate:methanol (7:3) step gradient mixtures as an eluent. Their structures were established by elemental analysis, IR, ^{1}H, ^{13}C, ^{31}P NMR, and mass spectral data (Tables 1–4).

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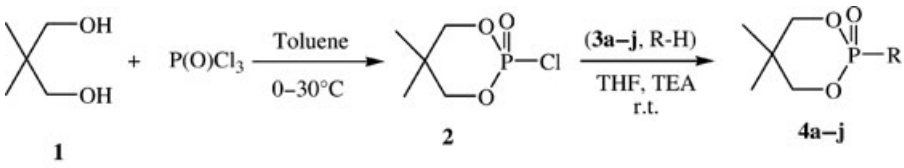

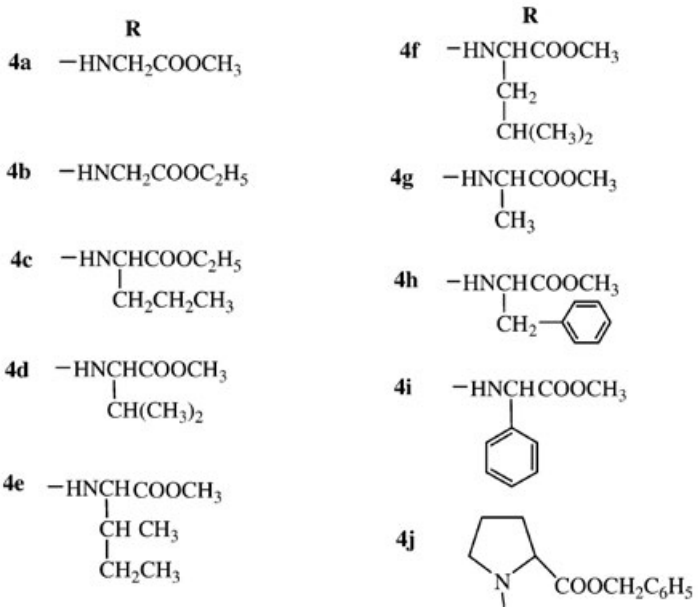

SCHEME 1

In the ^{1}H NMR spectra (Table 2), aromatic protons resonated at δ 6.91–7.90. The singlet at δ 10.20–11.34 is assigned to P-NH. ^{13}C NMR chemical shifts (Table 3) were assigned based on the reported literature data [9]. ^{31}P NMR spectra of **4a–j** showed only one phosphorus resonance signal in the range of –5.89 to 4.19 ppm [10].

ANTIMICROBIAL ACTIVITY

The compounds were diluted in dimethylformamide (DMF) for bioassay. Solvent control was included although no antimicrobial activity has been noted in the solvent employed. Ciprofloxacin controls were included to compare with compounds **4a–j**. All

TABLE 1 Physical, IR and ^{31}P NMR Spectral Data of **4a–j**

Compound	Melting Point (°C)	Yield (%)	Molecular Formula	Elemental Analysis Found (Calcd) (%)			IR[a] (cm^{-1})			31P NMR[b,c]
				C	H	N	P=O	C=O	NH	
4a	104–105	68	$C_8H_{16}NO_5P$	40.34 (40.49)	6.68 (6.74)	5.79 (5.90)	1213	1644	3410	2.36
4b	111–112	59	$C_9H_{18}NO_5P$	42.85 (43.01)	7.21 (7.16)	5.43 (5.57)	1213	1635	3407	2.69
4c	118–119	61	$C_{12}H_{24}NO_5P$	49.14 (48.96)	8.19 (8.14)	4.77 (4.71)	1216	1648	3418	2.91
4d	123–124	57	$C_{11}H_{22}NO_5P$	47.42 (47.28)	7.76 (7.88)	5.13 (5.01)	1210	1644	3418	4.19
4e	119–120	62	$C_{12}H_{24}NO_5P$	49.21 (49.14)	8.26 (8.19)	4.62 (4.77)	1211	1659	3413	4.24
4f	114–115	72	$C_{11}H_{22}NO_5P$	47.18 (47.31)	7.80 (7.88)	4.91 (5.01)	1217	1649	3415	2.23
4g	127–128	64	$C_9H_{18}NO_5P$	43.14 (43.01)	7.09 (7.16)	5.43 (5.57)	1214	1626	3431	2.03
4h	117–118	68	$C_{15}H_{22}NO_5P$	55.09 (55.01)	6.58 (6.72)	4.20 (4.27)	1215	1624	3410	–4.83
4i	116–117	71	$C_{14}H_{20}NO_5P$	53.69 (53.64)	6.31 (6.38)	4.38 (4.47)	1212	1648	3436	–4.62
4j	134–135	68	$C_{17}H_{24}NO_5P$	57.69 (57.76)	6.70 (6.79)	3.86 (3.96)	1210	1658	–	–5.89

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

TABLE 2 ^{1}H NMR Spectral Data[a,b] of **4a–j**

Compound	*CH_2 (4 and 6)*	*5,5-$(CH_3)_2$*	*N-H*	*Ar-H*	*Amino Acid Ester-H*
4a	3.95–4.03 (m, 4H)	1.02, 0.90 (2s, 6H)	11.25 (s, 1H)	–	3.15 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2)
4b	3.80–4.03 (m, 4H)	1.02, 0.90 (2s, 6H)	11.16 (s, 1H)	–	3.95 (m, 2H, OCH_2), 3.48 (s, 2H, CH_2), 3.14 (s, 3H, CH_3)
4c	3.81–4.16 (m, 4H)	1.06, 0.80 (2s, 6H)	11.24 (s, 1H)	–	3.92 (m, 2H, OCH_2), 4.51 (m, 1H, NH-CH-), 2.26 (m, 2H, $CH_2CH_2CH_3$), 2.16 (m, 2H, $CH_2CH_2CH_3$), 1.41 (t, $CH_2CH_2CH_3$)
4d	3.82–4.27 (m, 4H)	1.20, 0.90 (2s, 6H)	11.34 (s, 1H)	–	3.72 (s, 3H, OCH_3), 3.67–3.71 (m, 1H, CH-$(CH_3)_2$), 0.98 (d, 6H, $J = 12.7$ Hz, $CH(CH_3)_2$, 1.20–1.31 (m, 1H, CH-$CH(CH_3)_2$)
4e	3.87–4.24 (m, 4H)	1.18, 0.89 (2s, 6H)	11.10 (s, 1H)	–	3.60 (s, 3H, OCH_3), 4.07–4.11 (m, 1H, NH-CH), 3.07–3.19 (m, 1H-CH-$CH(CH_3)$-CH_2CH_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)
4f	3.81–4.20 (m, 4H)	1.17, 0.91 (2s, 6H)	10.20 (s, 1H)	–	3.26 (s, 3H, OCH_3), 4.46–4.52 (m, 1H, NH-CH), 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-$CH(CH_3)_2$
4g	3.61–3.95 (m, 4H)	1.24, 0.90 (2s, 6H)	11.24 (s, 1H)	–	3.17 (s, 3H, OCH_3), 3.72 (m, 1H, CH), 0.83 (d, 3H, CH_3)
4h	3.69–4.14 (m, 4H)	1.25, 0.90 (2s, 6H)	11.04 (s, 1H)	7.07–7.20 (m, 5H)	3.69 (s, 3H, OCH_3), 3.95 (d, 2H, CH_2), 4.14–4.41 (m, 1H, CH-CH_2)
4i	3.93–4.44 (m, 4H)	1.30, 0.88 (2s, 6H)	11.28 (s, 1H)	7.21–7.36 (m, 5H)	3.72 (s, 3H, OCH_3), 5.15 (s, 1H, $CHCOOCH_3$)
4j	3.78–4.25 (m, 4H)	1.17, 0.86 (2s, 6H)	–	7.27–7.36 (m, 5H)	5.12–5.20 (s, 2H, OCH_2), 4.07 (m, 1H, CH (C-2′), 1.94–2.08 (m, 4H, CH_2CH_2(C-3′, C-4′), 3.42 (m, 2H, CH_2 (C-5′)

[a]Recorded in $CDCl_3$
[b]Chemical shifts are in ppm.

TABLE 3 ^{13}C NMR Chemical Shifts of Some Members of **4**[a,b]

Compound	*C-5*	*C-4 and C-6*	*5,5-$(CH_3)_2$*		*Chemical Shifts (Amino Acid Ester)*
4a	31.6	78.4	21.8	19.3	173.1 ($COOCH_3$), 47.2 (CH_2COOCH_3), 57.1 (OCH_3)
4d	31.6	78.9	21.8	19.7	171.6 ($COOCH_3$), 45.9 ($CHCOOCH_3$), 57.4 (OCH_3), 29.6 ($CH(CH_3)_2$, 21.6, 21.1 ($CH(CH_3)_2$)
4h	31.8	79.0	21.8	19.8	172.3 ($COOCH_3$), 54.5 (OCH_3), 135.0 (s, 2C, C_3^1 and C_5^1), 127.1 (s, 2C, C_2^1 and C_6^1), 129.8 (s, 1C, C_4^1), 55.3 (CH-CH_2-C_6H_5)
4i	32.2	77.8	21.8	21.3	172.5 ($COOCH_3$), 52.7 (OCH_3), 55.3 ($CHCOOCH_3$), 129.8 (s, 2C, C_3^1 and C_5^1), 12.3 (s, 2C,C_2^1 and C_6^1), 135.0 (s, 1C, C_4^1
4j	31.8	79.1	21.8	21.2	172.0 ($COOCH_2C_6H_5$), 66.8 ($COOCH_2C_6H_5$) 58.3 (S, 1C, C_2), 31.8 (S, 1C, C_3), 24.3 (S, 1C, C_4) 46.0 (d, 1C, C_5), 135.4 (S, 1C, C_1^1, 128.7 (S, 2C, C_6^1) 127.4 (S, 2C, C_3^1 and C_5^1), 128.5 (S, 1C, C_4^1)

[a]Recorded in $CDCl_3$.
[b]Chemical shifts are in ppm.

TABLE 4 Mass Spectral Data of Important Ions of **4i** and **4j**

Compound	*m/z (Relative Abundance)*
4i	314 ($M^+ + 1$), 313 (M^+, 26) (12), 268 (18), 247 (14), 228 (8), 179 (18), 167 (30), 152 (18), 136 (16), 118 (6), 102 (79), 99 (16), 91 (12)
4j	354 ($M^+ + 1$, 100), 353 (M^+, 11), 352 (21), 315 (12), 306 (6), 273 (4), 262 (12), 234 (32), 218 (96), 216 (9), 150 (42), 136 (16), 102 (28)

samples were tested in triplicate, and average results were recorded.

The compounds were assayed for antibacterial activity against six registered bacterial isolates, which were obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratories, Pune, India. The antibacterial activity of all the title compounds **4a–j** was evaluated [11,12] against the growth of *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) at concentrations (100, 50, 25 ppm). The bacteria were rejuvenated on Hi-media nutrient agar and subcultured as needed.

The compounds **4a–j** (Table 5) were screened for their antifungal activity against *Aspergillus niger* and *Helminthosporium oryzae* along with the standard fungicide bavistin. The disk diffusion method [13] was followed for screening of the compounds at three different concentrations (25, 50, 100 ppm). Their antibacterial activity was also evaluated according to the disk diffusion method at three different concentrations against *E. coli* and *S. aureus* by comparing with standards streptomycin and penicillin.

Compounds **4a–j** were screened for their antibacterial activity against *E. coli* and *S. aureus* (10^6 cell/mL) by the disk diffusion method in nutrient agar medium at various concentrations (25, 50, 100 μg/disk) in dimethylformamide (DMF). The solutions were added to each filter disk, and DMF was used as the control. The plates were incubated at 35°C and examined for the zone of inhibition around each disk after 24 h. The results were compared with the activity of the standard antibiotic penicillin (25, 50, 100 μg/disk). Their antifungal activity was evaluated against *A. niger* and *H. oryzae* at concentrations of 25, 50, 100 μg/disk. Bavistin was used as a reference compound. Fungal cultures were grown on potato dextrose broth at 25°C, and finally spore suspension was adjusted to 10^5 spores/mL.

It is interesting to observe that all compounds **4a–j** exhibited more antifungal and antibacterial activity when compared with that of standard. The highlight is that all the compounds exhibited very high activity against fungi, and some compounds were twice more effective than the standard bavistin. Similarly, it is gratifying to observe that these compounds are extremely more effective against bacteria *S. aureus*.

TABLE 5 Antifungal and Antibacterial Activities of Compounds **4 a–j** in Terms of Zone of Inhibition (mm)

	Fungi						*Bacteria*					
	Aspergillus niger			*Heliminthosporium oryzae*			*Escherichia coli*			*Staphylococcus aureus*		
Compound	*100 (μg/disk)*	*50 (μg/disk)*	*25 (μg/disk)*	*100 (μg/disk)*	*50 (μg/disk)*	*25 (μg/disk)*	*100 (μg/disk)*	*50 (μg/disk)*	*25 (μg/disk)*	*100 (μg/disk)*	*50 (μg/disk)*	*25 (μg/disk)*
4a	12	9	–	12	6	–	13	8	5	11	8	5
4b	10	8	4	13	10	5	12	8	3	11	8	6
4c	10	8	5	12	11	7	11	9	5	12	9	5
4d	12	9	8	13	10	5	13	9	3	14	11	4
4e	9	5	3	11	9	6	8	3	–	10	7	–
4f	9	5	–	10	4	–	10	5	–	9	3	–
4g	14	9	6	12	10	8	12	9	3	11	8	5
4h	13	7	4	11	10	5	8	5	–	–	3	–
4i	13	9	6	11	8	6	9	4	–	12	10	–
4j	10	8	6	13	10	4	12	8	2	12	9	3
Bavistin	8	5	2	12	9	3	–	–	–	–	–	–
Penicillin	–	–	–	–	–	–	10	6	3	9	5	2

All concentrations are expressed in ppm.
– indicates no activity.

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).

*Synthesis of 2-Chloro-5,5-dimethyl[1,3, 2]dioxaphosphorinane 2-oxide (**2**)*

A solution of phosphorus oxychloride (0.76 g, 5 mmol) in 25 mL of dry toluene was added dropwise over a period of 20 min to a stirred solution (0.52 g, 5 mmol) of 2,2-dimethyl-1,3-propanediol (**1**) and triethylamine (1.01 g, 10 mmol) in 50 mL of toluene at 5–10°C. After the addition, the reaction mixture was stirred at room temperature for 4 h. Completion of the reaction was monitored by the 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°C) and used for the second step reactions without further purification.

*Synthesis of (5,5-Dimethyl-2-oxido-[1,3, 2]dioxaphosphorinane-2-yl)pyrrolidine-2(5)-benzyl carboxylate (**4j**)*

A mixture of proline benzyl ester hydrochloride (**3j**; 0.483 g, 2 mmol) and 2-chloro-5,5-dimethyl [1,3,2]dioxaphophorinane 2-oxide (**2**) (0.369 g, 2 mmol) and triethylamine (0.41 g, 4 mmol) in 50 mL of dry THF was stirred at room temperature for 6 h. The progress of the reaction was monitored by the TLC analysis. Triethylamine hydrochloride was separated by filtration, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel, using ethyl acetate: methanol (7:3) as an eluent, to yield 0.48 g (68%) of **4j**, mp 134–135°C. All the other compounds (**4a–j**) were prepared by adopting the above procedure.

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