SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF *N*-(SUBSTITUTED)-*N'*-[8-OXIDO DINAPHTHO-16*H*-(2,1-*D*:(1',2'-*G*)1,3,2-DIOXAPHOSPHOCIN-8-YL]UREAS

U. Anasuyamma, P. Haranath, B. Siva kumar and C. Suresh Reddy*

*Department of Chemistry, Sri Venkateswara University, Tirupati-517 502, India.
<csureshsvu@yahoo.com>
Department de Quimica Organica, Faculatad de Quimica, Universidad de Murcia, Campus de Espinardo, E-30100 Murcia, Spain

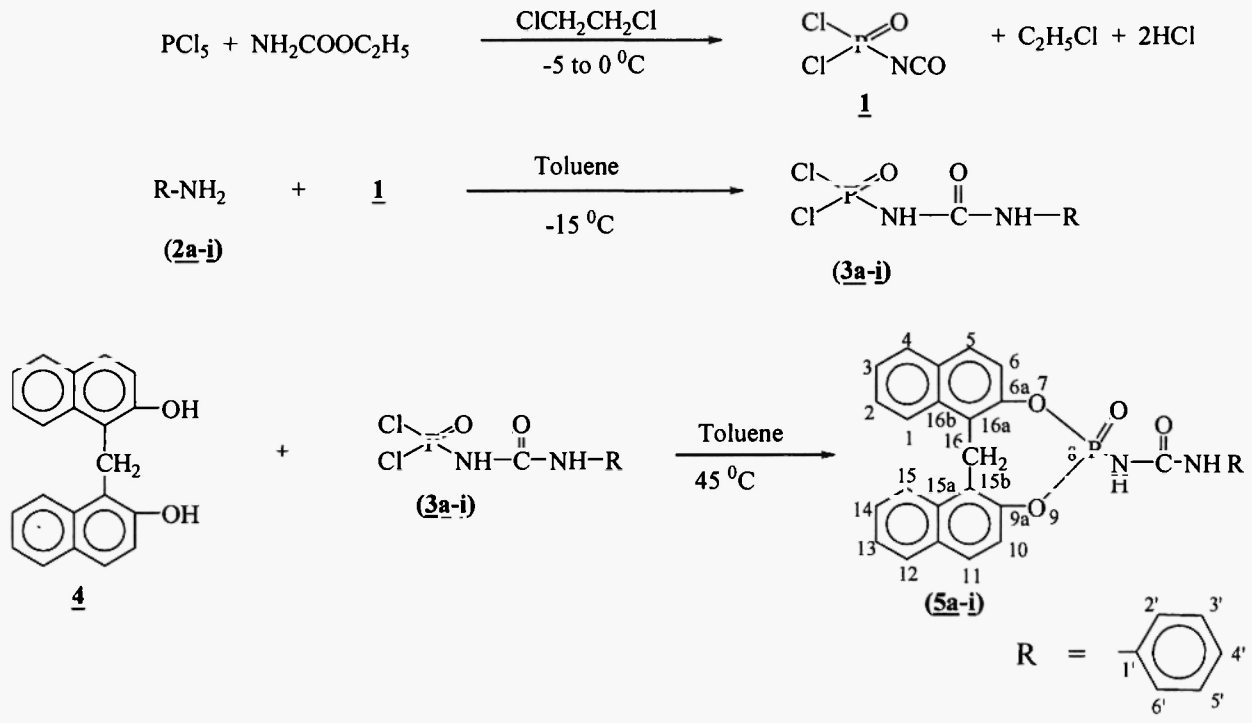
Abstract : Substituted dinaphtho-16*H*-(2,1-*d*.(1',2'-*g*)1,3,2-dioxaphosphocin-8-yl]ureas (**5a-i**) were synthesized by reacting bis (2-hydroxy-1-naphthyl)methane (**4**) with different carbamidophosphoric acid dichlorides (**3**) in the presence of triethylamine in dry toluene at 45-50 °C. Their structures were established by elemental analysis, IR, ^{1}H, ^{13}C & ^{31}P NMR spectral data. These compounds were found to possess good antimicrobial activity.

Introduction

Organophosphorus compounds being ubiquitous in nature have found multifaceted applications. Phosphorous heterocycles substituted with carbamate moieties are important classes of antitumour agents [1], pesticides [2], and bactericides [3, 4]. Substituted phosphoryl ureas of the type RR'P(O)NHCONR'' R''' exhibited pesticidal activity [5-7]. In view of this several, *N*-(substituted)-*N'*-[8-oxidodinaphtho-16*H*-(2,1-*d*: (1',2'-*g*)1,3,2-dioxaphosphocin-8-yl]ureas have been synthesized, expecting them to possess broad spectrum of biological activity and characterized by elemental, IR, NMR (^{1}H, ^{13}C and ^{31}P) spectral analyses.

Results and Discussion

The synthetic route (**Scheme-1**) involves the addition of dichloroisocyanato phosphine oxide [1, 8] (**1**) with various amines (**2a-i**) at -15°C under inert anhydrous conditions in dry toluene to afford the corresponding carbamidophosphoric acid dichlorides [9, 10] (**3a-i**). After the completion of addition of amines to **1**, the products separated from the reaction mixture immediately as crystalline compounds. Further purification of carbamidophosphoric acid dichlorides (**3a-i**) could not be accomplished due to their insolubility in many organic solvents and air sensitivity. Hence they were reacted directly *in situ* with a solution of bis (2-hydroxy-1-naphthyl)methane (**4**) in toluene in the presence of two equivalents of triethylamine to yield the compounds **5a-i** and their structures were established by IR, NMR (^{1}H, ^{13}C and ^{31}P) spectral data (Tables-1,2 and 3).



Compd.	R	Compd.	R
5a	C_6H_5	5f	C_6H_4-OCH_3(4')
5b	C_6H_4-CH_3(4')	5g	C_6H_3-$(OCH_3)_2$(3',4')
5c	C_6H_4-F(4')	5h	C_6H_4-CH_2Cl(2')
5d	C_6H_4-Cl(4')	5i	C_6H_{11}
5e	C_6H_4-Br(4')		

Scheme-1

The infrared spectral data (Table 1) of **5a-i** exhibited stretching frequencies in the region 1232-1259, 3302-3362 and 1664-1741 cm^{-1} for P=O, P-NH and C=O respectively [11, 12,13].

In the proton NMR spectral data (Table 2) of (**5a-i**), the aromatic protons resonated as multiplets at slightly downfield (δ 7.87-6.63) when compared to those of the starting compound **4** (δ 7.20-6.49) due to the deshielding effect of benzoxazaphosphorin 2-oxide ring. The chemical shifts of C-4 methylene protons appeared as two distinct doublets in the region δ 4.86-4.78 and 5.27-5.20($^2J_{H-H}$ = 16.2-16.5 Hz) indicating their non-equivalence and coupling with phosphorus in the eight-membered chair conformation of the dinaphthodioxaphosphocin 8-oxide system [13].

Table-1 : Synthetic and analytical data of *N*-(substituted)-*N'*-[8-oxido dinaphtho-16*H*-(2,1-*d*:(1',2'-*g*)1,3,2-dioxaphosphocin-8-yl]ureas

Compd.	mp (°C)	Yield (%)	Molecular formula	Elemental analysis Found (Calcd)%			IR(cm^{-1})			^{31}P NMR
				C	H	N	P=O	C=O	P-NH	
5a	241-243	71	$C_{28}H_{21}N_2PO_4$	70.18 (70.00	4.43 4.41	5.87 5.83)	1248	1732	3362	22.0
5b	142-144	68	$C_{29}H_{23}N_2PO_4$	70.40 (70.44	4.70 4.69	5.69 5.67)	1259	1715	3302	23.7
5c	147-149	62	$C_{28}H_{20}N_2PO_4F$	67.51 (67.47	4.07 4.04	5.67 5.62)	1232	1741	3321	24.6
5d	163-165	60	$C_{28}H_{21}N_2PO_4Cl$	65.37 (65.31	3.95 3.92	5.48 5.44)	1246	1726	3312	21.9
5e	146-148	63	$C_{28}H_{20}N_2PO_4Br$	60.19 (60.12	3.67 3.60	5.04 5.01)	1244	1732	3347	31.8
5f	144-146	66	$C_{29}H_{23}N_2PO_5$	68.29 (68.23	4.58 4.54	5.53 5.49)	1242	1714	3317	37.5
5g	131-133	64	$C_{30}H_{25}N_2PO_6$	66.70 (66.66	4.69 4.66	5.19 5.15)	1249	1720	3323	33.7
5h	179-181	70	$C_{29}H_{22}N_2PO_4Cl$	65.68 (65.85	4.24 4.19	5.39 5.30)	1253	1664	3311	25.6
5i	194 - 196	58	$C_{28}H_{27}N_2PO_4$	69.20 (69.13	5.55 5.59	5.79 5.76)	1256	1728	3344	31.6

Table-2 : ^{1}H NMR Spectral data[a,b] of *N*-(substituted)-*N'*-[8-oxidodinaphtho-16*H*(2,1-*d*:(1',2'-*g*)1,3,2-dioxaphosphocin-8-yl]ureas (5a-i)

Compd.	Ar-H	-C$\underline{H}_2$-(bridged)		-N$\underline{H}$C(O)	C(O)N$\underline{H}$-
		H_a	H_b		
5a	7.87-7.13 (m, 17H)	5.24 (16.4)	4.81 (16.5)	8.62	5.21
5b	7.71-7.10(m, 16H)	5.20 (16.2)	4.85 (16.4)	8.52	5.90
5c	7.52-6.63(m, 16H)	5.25 (16.4)	4.78 (16.4)	8.72	5.41
5d	7.41-6.69(m, 16H)	5.27 (16.4)	4.80 (16.4)	8.12	4.21
5e	7.81-7.04(m, 16H)	5.20 (16.4)	4.84 (16.3)	8.71	5.10
5f	7.61-6.99(m, 16H)	5.26 (16.4)	4.80 (16.4)	8.16	5.27
5g	7.23-6.84(m, 15H)	5.24 (16.4)	4.81 (16.4)	8.32	5.71
5h	7.73-7.20(m, 16H)	5.20 (16.4)	4.86 (16.4)	8.64	5.60
5i	7.61-6.87(m, 12H)	5.23 (16.4)	4.84 (16.2)	8.18	5.25

[a]Recorded in DMSO-d_6
[b]Chemical shifts in ppm

Table-3 : ^{13}C NMR Spectral data[a] [b] of some *N*-(substituted)-*N'*-[8-oxidodinaphtho-16*H*-(2,1-*d*:(1',2'-*g*)1,3,2-dioxaphosphocin-8-yl]ureas

Carbon	5a	5b	5c	5d	5e	5f	5g
C-1,15	127.3	128.9	126.7	127.7	126.1	127.1	127.9
C-2,14	125.8	125.4	124.4	124.4	125.9	126.2	127.6
C-3,13	125.4	124.3	124.3	123.4	125.4	124.7	126.4
C-4,12	129.4	129.3	129.1	129.3	129.2	129.8	129.1
C-5,11	129.1	129.0	127.6	129.1	127.7	129.6	128.2
C-6,10	120.4 (4.8)	120.4 (4.6)	120.4 (4.9)	120.5 (4.9)	120.5 (4.6)	120.9 (3.6)	120.4 (4.8)
C-6a,9a	148.6 (13.7)	148.5 (13.5)	148.5 (13.4)	148.6 (13.3)	148.7 (13.6)	149.1 (13.7)	148.6 (13.4)
C-15b/16a	124.4 (6.6)	123.3	123.4	121.4 (4.5)	123.5	122.4 (3.2)	120.6 (5.1)
C-15a/16b	132.2	132.2	132.2 (1.9)	132.5	132.3	132.7 (2.0)	132.9
C-4a/11a	132.6	132.4	132.5	132.6	133.0	136.1	132.5
C-16	24.2	24.2	24.3	24.3	24.4	24.8	24.3
C1'	148.7	148.8	148.7	148.6	148.8	150.1	147.7
C-2'	129.4	120.8	129.4 (1.2)	132.2 (1.9)	124.5	119.0 (4.6)	129.4
C-3'	131.6	130.2	127.5	124.3	123.5	127.0	125.8
C-4'	125.4	135.4	124.4	129.4	143.9	143.0	126.9
C-5'	127.6	130.2	124.3	127.5	123.5	127.0	123.7
C-6'	123.4	120.8	120.5	124.5	121.0	124.4	127.6
C=O	152.3	156.0	153.0	161.9	156.1	158.7	159.2
Alkyl	-	20.4	-	-	-	54.5	54.9 60.5

[a] Chemical shifts in ppm
[b] Recorded in DMSO-d_6

The signal of phosphorylamidic proton of P(O)-NH-C(O) appeared at extreme downfield, δ 8.72-8.12 when compared to that of carbamidic protons C(O)-NH-R resonance signal, δ 5.90-4.21. Absence of split signals for the protons of other groups (R) attached to the carbamido moiety shows that phosphorus coupling is limited to P-NH proton only [14].

The ^{13}C NMR chemical shifts (Table 3) for C-4 to C-10, C-1' to C-6' and C-1" to C-6" were observed in the expected range in the title compounds [15, 16]. However, the signals for the carbon of the carbamido function appeared at δ 161.9-152.3. Chemical shifts for other carbons were observed in the expected regions. The ^{13}C NMR chemical shifts could not be identified in the spectra of compounds **5f** and **5g** because of poor quality of the spectrum due to their meager solubility in DMSO.

The ^{31}P NMR signals of **5a-i** appeared as singlet [17] in the range of 21.9-37.5 ppm. (Table-1)

Antimicrobial Activity

The compounds **5a-i** (Table-4) were screened for their antifungal activity against *Aspergillus niger* and *Helminthosporium oryzae* by comparing with standard fungicide Bavistin. Disc diffusion method [18] was followed for screening the compounds at three different concentrations (25, 50, 100 ppm). Their antibacterial activity was evaluated according to the disc diffusion method [19, 20] at three different concentrations against *Escherichia coli* and *Staphylococcus aureus* by comparing with standards Streptomycin. The title compounds showed more antifungal activity when compared with antibacterial activity.

Compounds **5a-i** were moderate effective against *Staphylococcus aureus* and the compounds **5a-d, 5f-h** were effective against *Escherichia coli.* However all these compounds exhibited more antifungal activity against *Aspergillus niger* and *Helminthosporium oryzae.*

Experimental

Melting points were taken on Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by the Central Drug Research Institute, Lucknow, India. IR spectra were recorded as KBr pellets on a Perkin - Elmer 1430 unit. ^1H NMR and ^{13}C NMR spectra were recorded on AMX 400 MHz spectrometer operating at 400 MHz for ^1H, 100 MHz for ^{13}C and 161.9 MHz for ^{31}P. Compounds were dissolved in $CDCl_3$. The chemical shifts were referenced to TMS (^1H and ^{13}C) and 85% H_3PO_4 (^{31}P).

Bis (2-hydroxy-1-naphthyl)methane (**4**) was prepared by using the reported procedure [21].

Preparation of 4-chloro phenyl carbamido phosphoric acid dichloride (3d).

A solution of 4-chloro aniline (**2d**, 0.51 g, 4.0 mmol) in dry toluene (25 mL) was added dropwise (20 min) to a cold solution (-15°C) of isocyanatophosphoric dichloride (**1**, 0.64 g, 4.0 mmol) in dry toluene (30 mL). After the addition the temperature of the reaction mixture was maintained between -15°C to -5°C for 30-40 minutes. Later the reaction mixture was brought to room temperature and stirred for 30-40 minutes. 4-chlorophenyl carbamido phosphoric acid dichloride (**3d**) being insoluble in toluene separated

out. Without isolation and further purification, **3d** was used for cyclisation reactions. Other carbamidophosphoric acid dichlorides, **3a-i** were prepared following this procedure.

Synthesis of *N*-(substituted)-*N'*-[8-oxidodinaphtho-16*H*-(2,1-*d*:(1',2'-*g*)1,3,2-dioxaphosphocin-8-yl]urea (5d)

A solution of 4-chlorophenyl carbamidophosphoric acid dichloride (**2d**, 0.575 g, 2.0 mmol) in toluene (20 mL) was added to the solution of Bis(2-hydroxy-1-naphthyl)methane (**4**, 0.6 g, 2.0 mmol) and triethylamine (0.404 g, 4.0 mmol) in dry toluene (20 mL) at 0°C. After the addition, the reaction mixture was maintained at 0°C for one hour and then stirred at room temperature for further one hour. Later the temperature of the reaction mixture was raised slowly to 45-50 °C, with stirring for an additional five hours. The progress of the reaction was monitored by TLC in the 1:2 mixture of ethyl acetate and hexane as a mobile solvent and silica gel as adsorbent. Triethyl amine hydrochloride was separated by filtration and the solvent from the filtrate was evaporated under reduced pressure. The residue obtained after washing with water was triturated with isopropyl alcohol to afford 0.66 g (60%) of analytically pure material of **5d**, m.p 163-165 °C. Physical and spectral data of **5d** are given in Tables 1-3.

Other members of **5** were prepared by adopting the same procedure.

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Table-5 : Antifungal and Antibacterial activities of compounds 5 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
5a	7	-	-	10	6	-	9	5	-	9	4	-
5b	8	4	2	12	9	4	10	8	3	10	6	2
5c	9	6	2	12	7	-	11	8	3	12	7	2
5d	9	6	-	10	8	-	10	7	-	10	6	3
5e	10	6	2	10	6	2	-	-	-	10	6	-
5f	9	6	2	11	8	-	10	5	-	10	6	2
5g	10	6	2	12	7	-	12	8	-	10	7	-
5h	9	5	-	11	6	2	8	5	-	8	5	-
5i	10	4	-	12	8	2	-	-	-	11	8	3
Bavistin	8	5	-	12	9	-						
Streptomycin							10	6	-	9	5	-

Concentrations expressed in ppm

'-' indicates no activity

References

1. Zhadanov, RI. Buina, WA. Kapitanova, NA & Nuretdinov, IA, *Synthesis,* 269, (1979).
2. Fest, C & Schmidt, KJ, The Chemistry of Organophosphorus Pesticides (Springer-werlag, Berlin), 1982.
3. Bhatia, MS. & Jit, P, *Experientia.,* **32**, 1111 (1976).
4. Ismail, R, Ger pat, 1543539, (1975), *Chem Abstr.,* **83**, 974116q. (1975).
5. Kirsanov, A.V, *Zhur Obshchei Khim.,* **24**, 1033 (1954), *Chem Abstr.,* **49**, 8787 b (1955).
6. Kisanov, A.V. & Zhmurova, I.W, *Zhur Obshchei Khim.,* **28**, 2478 (1958), *Chem Abstr.,* **53**, 3118i (1959).
7. Kirsanov, A.V. & Marenets, M.S. *Zhur Obshchei Khim.,* **29**, 2256 (1959) *Chem Abstr.,* **54**, 10855e (1960).
8. Papanastassiou, Z.B. & Bardos, T.J, *J. Med. Chem.,* **5**, 1000 (1962).
9. Nagaprasada Rao, L, Devendranath Reddy, C & Sankara Reddy, B, *Indian J. Chem.,* **40B**, 817 (2001).
10. Kirsanov, A.V & Levchenko, E.S, *J. Gen. Chem.,* U.S.S.R, **26**, 2555 (1956).
11. Thomas, L.C &. Chittenden, R.A, *Chem Soc* (London), 1913 (1961).
12. Thomas, L.C, The Interpretation of the Infrared Spectra of Organophosphorus Compounds, Heydon-London, (1974).
13. Reddy, C.D, Reddy, R.S, Reddy M.S, Krishnaiah, M, Berlin, K.D & Sunthankar, P, *Phosphorus Sulfur Silicon,* **62,** 1 (1991).
14. Silverstein, R.M. & Webster, F.X, Spectrometric Identification of Organic Compounds, 6th ed., Wiley, New York, 1998.
15. Reddy, C.D, Reddy, R. S, Raju, C.N, ElMasri, M, Berlin, K.D & Subramanian, S, *Magn Reson. Chem.,* **29**, 1140 (1991).
16. Reddy, C.D, Berlin, K.D, Reddy, R.S, Raju, C.N, ElMasri, M.& Subramanian, S, *Phosphorus Sulfur Silicon,* **81**, 61 (1993).
17. Quin, L.D, & Verkade, J.G, Phosphorus-31 NMR Spectral Properties in Compound Characterization and Structural Analysis, VCH Publisher, Inc, New York, 1994.
18. Benson, H.J, Microbiological Applications, Wm. C. Brown Publications, 5th ed., USA, 134(1990).
19. Cruickshan, K.R, Medical Microbiology, A guide to Diagnosis and Control of Infection, II Edition, E & S Livingston Ltd., (London) 888 (1968).
20. Beuer, A.W, Kirby, M.M, Sherries, J.C & Truck, A, *Am. J. Clin. Pathol.,* **45**, 493 (1969).
21. Ogata, Y. & Kowasaki, A, Tetrahedron, **25**, 2589, 1969.

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