Heteroatom Chemistry
Volume 14, Number 6, 2003

Synthesis and Antimicrobial Activity of *N*-Substituted *N′*-[6-Methyl-2-oxido-1,3,2-dioxaphosphinino(5,4-*b*)pyridine-2-yl]ureas

P. Vasu Govardhana Reddy, C. Suresh Reddy, and M. Venugopal

Department of Chemistry, Sri Venkateswara University, Tirupati 517 502, India

Received 21 March 2003

ABSTRACT: *N-Substituted N′-[6-methyl-2-oxido-1,3,2-dioxaphosphinino(5,4,-b)pyridine-2-yl]ureas have been accomplished by condensation of equimolar quantities of chlorides of various carbamidophosphoric acids (**3**) with 3-hydroxyl-6-methyl-2-pyridinemethanol (lutidine diol) (**4**) in the presence of triethylamine in dry toluene–tetrahydrofuran (1:1) mixture at 45–50°C. Their structures were established by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectral data. Their antifungal and antibacterial activity is also evaluated. Most of these compounds exhibited moderate antimicrobial activity in the assays.* © 2003 Wiley Periodicals, Inc. Heteroatom Chem 14:509–512, 2003; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.10181

INTRODUCTION

Organophosphorus carbamates demonstrated insecticidal, bactericidal, antiviral, and antitumor activity [1–5]. Industrially they were found to be useful as lubricating oil additives, antioxidants, and polymer stabilizers [6]. Pyridine-annulated cyclophosphamide was reported to have promising anticancer activity [7,8]. In our attempt to synthesize potential antimicrobial compounds, we reported earlier synthesis of some 1,3,2-dioxaphosphepino-pyridine-9-ol-3-oxides and 6-methyl-1,3,2-dioxaphosphinino-(5,4-*b*)pyridine 2-oxides [9,10]. In pursuit of the same aim, presently we report the synthesis of title compounds, their spectral data, and biological activity.

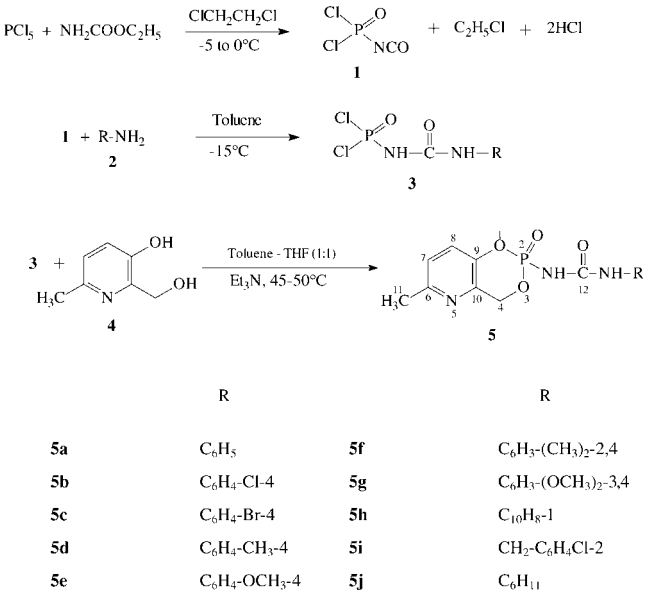
RESULTS AND DISCUSSION

The synthetic route (Scheme 1) involves the addition reaction of isocyanato phosphonic dichloride (**1**) [2,3] with various amines (**2a–j**) at −15°C under inert anhydrous conditions in dry toluene to afford the corresponding chlorides of carbamidophosphoric acids (**3a–j**) [11,12]. The reaction products separated from the reaction mixture immediately as crystalline compounds, after complete addition of amines. Further purification of carbamidophosphoric acids (**3)** could not be achieved because of their insolubility in many organic solvents and their air sensitiveness. Hence they were directly reacted with a solution of lutidine diol (**4**) in tetrahydrofuran in the presence of 2 equiv. of triethylamine to afford **5a–j**.

The IR spectra of **5a–j** exhibited characteristic bands [13,14] in the regions 3203–3327 (P—NH), 1220–1265 (P=O), and 1636–1683 (C=O) cm^{-1}. Reaction yields, elemental analyses, and ^{31}P NMR data of compounds **5a–j** are given in Table 1.

In ^{1}H NMR spectra of **5a–j** [15] (Table 2), the C-4 methylene protons of the heterocyclic ring resonated as a multiplet because of their coupling with phosphorus in the region δ 3.97–5.26. The multiplets at δ 6.66–8.25 are due to the aromatic protons. The signal

Correspondence to: C. Suresh Reddy; e-mail: csureshsvu@yahoo.com, csureshsvu@rediffmail.com.
Contract grant sponsor: CSIR, New Delhi.
Contract grant sponsor: UGC, New Delhi.



SCHEME 1

of phosphorylamidic proton of P(O)—NH—C(O) appeared in extreme downfield at δ 8.13–9.18, when compared to the carbamidic proton C(O)—NH—R resonance signal, δ 5.28–6.64. Absence of split signals for the other protons of carbamido moiety shows that phosphorus coupling is limited to P—NH protons only. The NH proton signals were confirmed by D_2O exchange experiments.

The ^{13}C NMR chemical shifts (Table 3) were recorded for some members of the title compounds. A low intense downfield signal in the region δ 146.26–151.34 ppm was assigned to C-6, which is deshielded because of nitrogen and the attachment of methyl group. C-9 of the pyridine ring resonated in the region δ 137.70–143.14 ppm. The C-10 also gave signal at the downfield at δ 142.21–147.11 ppm because it is ortho to nitrogen. The C-11 methyl group resonated as a singlet in the region δ 21.39–23.47 ppm. The C-4 methylene signal of the heterocycle appeared at δ 52.97–57.21. The signal of C-12 of the carbamido function resonated at δ 150.06–154.97 ppm. The chemical shifts could not be identified in the ^{13}C NMR of **5c, 5g, 5h, 5i**, and **5j** because of the poor quality of the spectrum owing to their meager solubility in DMSO.

The ^{31}P NMR signals [16] for **5** appeared in the region δ −11.43 to 14.24 ppm from 85% H_3PO_4.

ANTIMICROBIAL ACTIVITY

All the compounds **5a–j** (Table 4) were screened at two different concentrations (250 and 500 ppm) for their antifungal activity on *Aspergillus niger* and *Helminthosporium oryzae*. Griseofulvin was used as

TABLE 1 Physical, IR, and ^{31}P NMR Data of Compounds **5**

				Analysis Found (Required) (%)			*IR (cm^{-1})*			
	m.p. (°C)	*Yield (%)*	*Mol. Formula*	*C*	*H*	*N*	*C=O*	*P=O*	*P–NH*	*δ $^{31}P^{a,b}$*
5a	241–243	61[c]	$C_{14}H_{14}N_3O_4P$	52.36 (52.67)	4.68 (4.41)	12.89 (13.16)	1646	1239	3225	1.80, 3.90
5b	142–144	58[c]	$C_{14}H_{13}ClN_3O_4P$	47.26 (47.54)	3.52 (3.70)	11.57 (11.88)	1667	1245	3236	−51.6, −4.51
5c	147–149	62[c]	$C_{14}H_{13}BrN_3O_4P$	42.02 (42.23)	3.53 (3.29)	10.36 (10.55)	1678	1239	3258	–
5d	163–165	60[c]	$C_{15}H_{16}N_3O_4P$	54.31 (54.05)	4.56 (4.83)	12.32 (12.58)	1663	1259	3203	−9.16, −7.85
5e	146–148	54[c]	$C_{15}H_{16}N_3O_5P$	51.82 (51.58)	4.33 (4.61)	12.29 (12.03)	1683	1239	3289	−5.84, −5.51
5f	144–146	56[c]	$C_{16}H_{18}N_3O_4P$	54.56 (55.33)	4.98 (5.22)	12.32 (12.09)	1636	1265	3218	−8.14, −7.34
5g	131–133	52[c]	$C_{16}H_{18}N_3O_6P$	50.38 (50.66)	4.59 (4.78)	11.34 (11.07)	1654	1252	3263	−5.76, −4.26
5h	179–181	50[c]	$C_{17}H_{16}N_3O_4P$	57.46 (57.14)	4.72 (4.51)	11.58 (11.76)	1669	1220	3239	–
5i	94–96	48[c]	$C_{16}H_{15}ClN_3O_4P$	50.28 (50.60)	3.74 (3.98)	11.29 (11.06)	1635	1248	3276	2.80, 14.24
5j	106–108	43[c]	$C_{14}H_{20}N_3O_4P$	51.42 (51.69)	6.47 (6.19)	12.67 (12.92)	1678	1242	3327	−11.43, 0.31

[a] ^{31}P NMR chemical shifts were expressed in δ from 85% H_3PO_4 as external standard.
[b] Recorded in DMSO-d_6.
[c] Triturated from hot methanol.

TABLE 2 ^{1}H NMR Spectral Data of Compounds **5** (δ from TMS)[a]

	Ar-H (7-H, 8-H and R)	*–CH$_2$–*	*6-CH$_3$*	*R-CH$_3$/R-OCH$_3$*	*Cyclohexyl/ CH$_2$-Ph*	*–NHCO*	*CONH–*
5a[b]	6.95–7.58 (m, 7H)	4.94–5.02 (m, 2H)	2.40 (s, 3H)	–	–	8.47 (s, 1H)	5.79 (s, 1H)
5b[b]	6.70–7.43 (m, 6H)	4.28–4.74 (m, 2H)	2.52 (s, 3H)	–	–	8.76 (s, 1H)	5.92 (s, 1H)
5c[b]	6.92–7.46 (m, 6H)	4.46–4.81 (m, 2H)	2.51 (s, 3H)	–	–	–	–
5d[c]	6.74–7.25 (m, 6H)	4.53–4.70 (m, 2H)	2.51 (s, 3H)	2.12 (s, 3H)	–	–	5.29
5e[b]	7.14 (d, 6.9, 1H) 7.04 (d, 8.0, 1H) 6.66–6.86 (m, 4H)	4.18–4.41 (m, 2H)	2.46 (s, 3H)	3.53 (s, 3H)	–	–	–
5f[b]	6.77–7.34 (m, 5H)	4.35–4.53 (m, 2H)	2.39 (s, 3H)	2.28 (s, 3H) 2.34 (s, 3H)	–	8.75 (s, 1H)	5.28 (s, 1H)
5g[b]	6.89–7.32 (m, 5H)	4.42–4.68 (m, 2H)	2.46 (s, 3H)	3.52 (s, 3H) 3.56 (s, 3H)	–	–	–
5h[b]	7.45–8.25 (m, 9H)	4.44–4.96 (m, 2H)	2.51 (s, 3H)	–	–	9.18 (s, 1H)	6.64 (d, 7.5, 1H)
5i[b]	7.09–7.65 (m, 6H)	3.97–4.31 (m, 2H)	2.45 (s, 3H)	–	4.51 (s, 2H)	8.13 (s, 1H)	6.62 (s, 1H)
5j[b]	7.17 (d, 8.1, 1H) 7.07 (d, 7.9, 1H)	5.12–5.26 (m, 2H)	2.40 (s, 3H)		1.18–1.74 (m, 11 H)	8.35 (s, 1H)	6.60 (s, 1H)

[a]Data in parentheses are coupling constants *J* in Hz.
[b]Recorded in DMSO-d_6.

standard according to the Benson technique [17]. Most of the compounds exhibited significant toxicity against both the fungi. Their antibacterial activity was evaluated following the method of Vincent and Vincent [18] on *Escherichia coli* and *Staphylococcus aureus*, using pencillin and tetracycline as standards.

EXPERIMENTAL

All melting points were determined on a 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. ^{1}H NMR and ^{13}C NMR spectra were recorded on AMX 400 MHz spectrometer operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C, and 161.9 MHz for ^{31}P. Compounds were dissolved in DMSO-d_6. The chemical shifts were referenced to TMS (^{1}H and ^{13}C) and 85% H_3PO_4 (^{31}P).

3-Hydroxy-6-methyl-2-pyridinemethanol (**4**, lutidine diol) was procured from Aldrich Chemical and was used without recrystallization.

TABLE 3 ^{13}C NMR Spectral Data of Compounds **5** of Sufficient Solubility on DMSO-d_6 (δ from TMS)

	5a	**5b**	**5d**	**5e**	**5f**
C-4	56.24	52.97	57.21	56.18	55.33
C-6	148.28	146.26	150.26	149.68	151.34
C-7	126.36	125.95	126.42	123.48	127.58
C-8	130.12	128.35	128.97	127.61	131.21
C-9	139.27	137.70	139.50	143.11	143.14
C-10	142.68	142.21	144.21	147.11	146.33
C-11	21.62	21.39	22.06	21.87	23.47
C-12	153.67	150.06	152.89	153.38	154.97
C-1 (R)	145.72	142.21	143.64	143.11	143.14
C-2 (R)	115.91	116.39	116.18	114.60	124.44
C-3 (R)	129.06	128.35	130.22	114.10	130.84
C-4 (R)	119.84	123.62	127.64	149.68	128.31
C-5 (R)	128.68	128.05	131.18	114.10	125.32
C-6 (R)	114.22	117.88	115.57	114.60	113.51
R^4-CH_3	–	–	20.46	–	21.83
R^2-CH_3	–	–	–	–	20.66
R^4-OCH_3	–	–	–	55.15	–

*Preparation of 4-Chlorophenyl Carbamidophosphoric Dichloride (**3b**)*

A solution of 4-chloro aniline (**2b**, 0.51 g, 0.004 mol) in dry toluene (25 ml) was added dropwise (20 min) to a cold solution (−15°C) of **1** (0.64 g, 0.004 mol) in dry toluene (30 ml). After the addition the temperature of the reaction mixture was maintained in between −15 and −5°C for 30–40 min. Later the temperature of the mixture was raised to room temperature, with stirring for 30–40 min. Compound **3b** being insoluble in toluene separated out. It was collected by filtration and dried under reduced pressure.

*Synthesis of N-Chlorophenyl-N′-[6-methyl-2-oxido-1,3,2-dioxaphosphinino(5,4-b)pyridine-2-yl]urea (**5b**)*

A solution of **3b** (0.575 g, 0.002 mol) in toluene (20 ml) was added to the solution of **4** (0.278 g, 0.002 mol) and triethylamine (0.404 g, 0.004 mol) in dry tetrahydrofuran (20 ml) at 0°C. It was kept for 1 h and then after 1 h of stirring at room temperature, the temperature of the reaction mixture was allowed to rise slowly to 45–50°C, and stirring

TABLE 4 Antifungal and Antibacterial Activity of Compounds **5**

	Zone of Inhibition (mm)							
	Fungi				*Bacteria*			
	Aspergillus niger		Helminthosporum oryzae		Escherchia coli		Staphylococcus aureus	
	250 ppm	*500 ppm*	*250 ppm*	*500 ppm*	*250 ppm*	*500 ppm*	*250 ppm*	*500 ppm*
5a	7	9	10	14	6	9	7	10
5b	10	13	9	11	8	10	10	14
5c	9	12	8	12	10	12	6	11
5d	8	10	6	9	4	6	–	–
5e	7	11	10	15	–	–	–	–
5f	10	16	7	8	–	–	–	–
5g	7	9	9	12	6	8	5	9
5h	8	10	12	15	–	–	3	5
5i	10	14	11	16	7	9	6	8
5j	6	9	7	11	4	6	5	7
Penicillin					20		24	
Tetracycline					28		32	
Griseofulvin	34		34					

–Indicates no activity.

was continued for an additional 5 h. The progress of the reaction was monitored by TLC in the 1:2 mixture of ethylacetate and hexane as eluent and silicagel as adsorbent. Triethylamine 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 hot methanol to afford 0.41 g (58%) of pure **5b**, m.p. 142–144°C. Physical and spectral data of **5b** are given in Tables 1–3. Other members of **5** are prepared by the same procedure.

ACKNOWLEDGMENTS

The authors express their thanks to Professor C. Devendranath Reddy and Dr. C. Naga Raju for their helpful guidance and discussions and the Directors of SIF, IISC, Bangalore, for spectral data. They also thank Md. Khamar Jahan, Research Scholar, Department of Botany, S.V. University, Tirupati, for her help in antimicrobial studies.

REFERENCES

[1] Kuranari, M. Japan Patent 26819, 1965; Chem Abstr 1966, 64, 9737h.
[2] Zhadanov, R. I.; Buina, W. A.; Kapitanova, N. A.; Nuretdinov, I. A. Synthesis 1979, 269.
[3] Papanastassiou, Z. B.; Bardos, T. J. J Med Chem 1962, 5, 1000.
[4] Friedman, O. M.; Papanastassiou, Z. B.; Levi, R. S. J Med Chem 1963, 6, 82.
[5] Arnold, H.; Bourseaux, F.; Brock, N. Arzeimittel Forsch 1961, 11, 143.
[6] Spivack, J. D. Brit Patent 1,087,399, 1982; Chem Abstr 1982, 97, 198374.
[7] Hill, D. L. A Review of Cyclophosphamide; Charles C. Thomas: Springfield, IL, 1975.
[8] Sosnovsky, G.; Buddha, D. P. Z Naturforsch 1983, 38b, 1146.
[9] Reddy, C. D.; Reddy, R. S. N.; Raghu, K. V.; Naga Raju, C. J Indian Council Chem 1994, 169.
[10] Devendranath Reddy, C.; Reddy, M. S.; Naga Raju, C. Indian J Chem 2000, 39B, 426.
[11] Kirsanov, A. V.; Levechenko, E. S. J Gen Chem USSR 1956, 26, 2555.
[12] Naga Prasada Rao, L.; Devendranath Reddy, C.; Sankara Reddy, B. Indian J Chem 2001, 40B, 817.
[13] Thomas, L. C.; Chittenden, R. A. Chem Soc (London) 1961, 1913.
[14] Thomas, L. C. The Interpretation of the Infrared Spectra of Organophosphorus Compounds; Heydon: London, 1974.
[15] Silverstein, R. M.; Webster, F. X. Spectrometric Identification of Organic Compounds, 6th ed., Wiley: New York, 1998.
[16] Quin, L. D.; Verkade, J. G. Phosphorus-31 NMR Spectral Properties in Compound Characterization and Structural Analysis; VCH Publishers: New York, 1994.
[17] Benson, H. J. Microbiological Applications; WC Brown Publications: USA, 1990; p. 134.
[18] Vincent, J. C.; Vincent, H. W. Proc Soc Expt Biol Med 1944, 55, 162.