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Synthesis, Characterization, and Biological Activity of Organophosphates Derived from Substituted Benzoxazole

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ABSTRACT: *The reaction of phosphorus oxychloride/phosphorus thiochloride with 2-(2′-mercaptophenyl)benzoxazole in 1:1, 1:2, and 1:3 molar ratio in the presence of stoichiometric amounts of triethylamine has afforded a series of organophosphates. These organophosphates have been characterized by elemental analyses, infrared,* ^{1}H *NMR,* ^{31}P *NMR, and mass spectral studies. The antifungal activity of these organophosphates has been evaluated against pathogenic fungi* Aspergillus niger *and* Fusarium oxysporium. *The antifungal screening data reveal that these compounds are more fungitoxic than 2-(2′-mercaptophenyl) benzoxazole. These organophosphates were also found to be insecticidal when tested against* Periplenata americana. © 2009 Wiley Periodicals, Inc. Heteroatom Chem 20:309–315, 2009; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20551

INTRODUCTION

Heterocyclic compounds were involved at the very beginning of life in the genesis of DNA and have played an essential role in many living systems. The nucleic acid bases are derivatives of the aromatic N-heterocycles pyrimidine and purine. Being a heterocyclic compound, benzoxazole is structurally related to biologically important bases and are used in research as a starting material for the synthesis of bioactive compounds [1,2]. Benzoxazole has been used in household as cosmetics and medicine. It is found within the chemical structures of pharmaceutical drugs such as "flunoxaprofen." The aromaticity of benzoxazole makes it stable. Although as a heterocycle, it has reactive sites that allow for functionalization. Benzoxazole derivatives are used primarily as antibacterial and antifungal agents [3,4].

Organophosphates are used in agriculture, in the home, in gardens, and in veterinary purposes [5–8]. Organophosphates exert their biological action on insects and mammals by attacking the neural transmission system and inhibiting the function of acetylcholinesterase enzyme [9]. Organophosphorus compounds containing a heterocyclic moiety increase the protonation at the site of pesticides and enhance their biological activity [10]. The numerous applications of organophosphates promoted us to undertake the synthesis of phosphorylated/thiophosorylated derivatives of 2-substituted benzazoles [11,12]. In continuation of our previous work on phosphorylated/thiophosorylated derivatives of 2-substituted benzazoles [13–16], we report herein the synthesis, characterization, and biological activity of organophosphates derived from 2-substituted benzoxazole.

RESULTS AND DISCUSSION

The reactions of phosphorus oxychloride ($POCl_3$)/phosphorus thiochloride ($PSCl_3$) with

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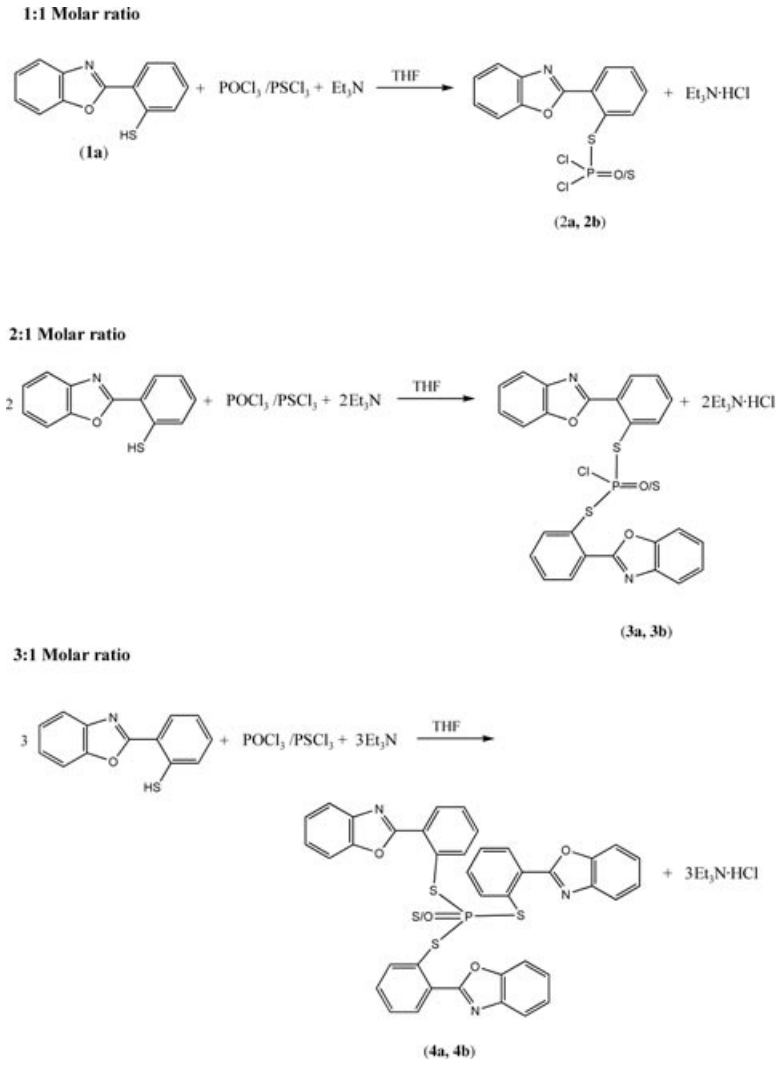

FIGURE 1

2-(2′-mercaptophenyl)benzoxazole in 1:1, 1:2, and 1:3 molar ratio in the presence of stoichiometric amounts of triethylamine in dry tetrahydrofurane (THF)/CH_2Cl_2 yielded the phosphorylated and thiophosphorylated benzoxazole derivatives **2a–b, 3a–b**, and **4a–b** (Table 1). A schematic presentation of these reactions is given in Fig. 1.

All these reactions are quite facile and completed in 12–14 h of refluxing. The resulted compounds are hygroscopic that are soluble in dimethylformamide and dimethylsulfoxide.

IR Spectra

The tentative assignments of some important bands are summarized in Table 2. The absorption band of medium intensity at 2570 cm^{-1}, due to the —SH group of 2-(2′-mercaptophenyl)benzoxazole, disappeared in the spectra of organophosphates (**2a–b, 3a–b, 4a–b**), suggesting the deprotonation of the —SH group and the formation of P—S—C bonds. These gets support by the appearance of new bands in the region 585–542 cm^{-1} due to νP—S—C, 1265–1250 cm^{-1} due to νP=O, 815–812 cm^{-1} and 700–690 cm^{-1} due to νP=S (I), and νP=S(II) bonds, respectively [17]. The organophosphates (**2a–b**) exhibit absorption bands at ~530–510 cm^{-1} and ~610–600 cm^{-1} due to ν_s (P—Cl) and ν_{as} (P—Cl) vibrations, respectively [18].

^{1}H NMR and ^{13}P NMR Spectra

The characteristic signals in ^{1}H NMR and ^{31}P NMR spectra of 2-(2′-mercaptophenyl) benzoxazole (**1a**) and organophosphates (**2a–b, 3a–b, 4a–b**) are summarized in Table 3. The ^{1}H NMR spectrum of **1a** shows —SH (thiophenolic) proton signal appeared at δ4.54 ppm and aromatic protons appeared at

TABLE 1 Synthetic and Analytical Data of **1a** and Organophosphates (**2a–b, 3a–b**, and **4a–b**) Derived from 2-(2′-Mercaptophenyl)benzoxazole

Compound	M. Wt. (Calcd) Found	m.p. (°C)	Yield (%)	Color	Analysis (%) (Calcd)/Found C	H	N	P	Cl	S
1a	(227.28) 234.03	185	66	Light brown	(68.70) 68.62	(3.99) 3.92	(6.16) 6.09	(14.10) 14.02	–	(14.10) 14.02
2a	(344.15) 343.01	205	53	Greenish cream	(45.37) 45.28	(2.34) 2.27	(4.07) 4.02	(9.00) 8.94	(20.60) 20.54	(9.32) 9.28
2b	(360.21) 358.99	207	49	Brownish green	(43.35) 43.22	(2.24) 2.12	(3.89) 3.76	(8.60) 8.48	(19.68) 19.54	(17.80) 17.65
3a	(534.97) 532.99	208	58	Greenish brown	(58.37) 58.22	(3.01) 2.88	(5.24) 5.12	(5.79) 5.68	(6.63) 6.48	(11.98) (11.86)
3b	(551.03) 550.14	210	55	Brownish green	(56.67) 56.62	(2.93) 2.82	(5.08) 4.96	(5.62) 5.48	(6.43) 6.28	(17.45) 17.27
4a	(725.79) 723.96	210	58	Greenish brown	(64.54) 64.38	(3.33) 3.18	(5.79) 5.66	(4.27) 4.16	–	(13.25) 13.12
4b	(741.85) 740.02	215	54	Brownish green	(63.14) (63.02)	(3.26) 3.15	(5.66) 5.54	(4.18) 4.02	–	(17.19) 17.16

δ6.8–7.8 ppm. The absence of —SH (thiophenolic) proton signal in these organophosphates indicates deprotonation of the thiophenolic group of **1a** and formation of the P—S—C bonds. All these organophosphates show multiplets in the region δ6.9–8.0 ppm attributable to the aromatic protons. The ^{31}P NMR spectra of these organophosphates exhibit signals at δ62.5–76.5 ppm, indicating the presence of a tetracoordinated phosphorus atom [19,20].

Mass Spectra

Mass spectra were recorded for determining the molecular mass of the organophosphates. The splitting pattern of the mass spectrum of compound $(C_{13}H_8NOS)P(O)Cl_2$ (**2a**) is represented in Fig. 2. The molecular ion peak appears at *m/z* 342.16, thus confirming the formation of compound **2a**. This peak after removal of two chlorine radicals generates the ion at *m/z* 272.02. It may further lose the radical PO, and a peak appears at *m/z* 225.1. After removal of a sulfur radical, a peak appears at *m/z* 192.18, which splits off a phenyl moiety producing an ion at *m/z* 115.14. It may further lose the ion at *m/z* 102. This peak after removal of a cyanide molecule generates the ion at *m/z* 77. Relative abundance of the ions is represented in Table 4.

Antifungal Activity

The results of antifungal activity of **1a** and organophosphates (**2a–b, 3a–b**, and **4a–b**) are presented in Table 5. The antifungal screening data reveals that these organophosphates are more fungitoxic than **1a**. The enhanced activity of these organophosphates may be ascribed to the increased lipophilic nature of these compounds. It is also noted that sulfur-containing compounds are more active than their oxygen-containing counterparts. The fungicidal activity increased as the concentration was increased.

Insecticidal Activity

The results of the insecticidal activity of **1a** and organophosphates (**2a–b, 3a–b**, and **4a–b**) have been compared with conventional insecticide Malathion, taken as standards (Table 6). The results reveal that these organophosphates are more toxic than **1a**, and their insecticidal activity increases with the increase in the concentration. Organophosphates (**2b, 3b**, and **4b**) having P=S bond resulted in higher toxicity than **2a, 3a**, and **4a** having P=O bond, with the same substituents attached to phosphorus.

TABLE 2 IR Spectral Data (cm^{-1}) of (1a) and Organophosphates (**2a–b, 3a–b**, and **4a–b**)

Compound	*ν(S–H)*	*ν(P–S–C)*	*ν(P=O)*	*ν(P=S)*	*ν(P–Cl)*
1a	2570				
2a		585	1265		590 (asymmetric)
					518 (symmetric)
2b		565			600 (asymmetric)
		545		690 (II)	510 (symmetric)
3a		575	1260		510
		545			
3b		542			495
				680 (II)	
4a		565	1250		
4b		560			
				700 (II)	

EXPERIMENTAL

All the chemicals used were of reagent grade. Phosphorus oxychloride ($POCl_3$)/phosphorus thiochloride ($PSCl_3$) were purchased from Fluka. All the solvents were dried and distilled before use. Melting points were determined by the capillary method and are uncorrected. All operations involving phosphorus compounds were carried out in dry equipment under nitrogen atmosphere. The IR spectra of all the compounds were recorded as KBr disks on a Shimadzu 8400 S FTIR spectrophotometer in the range 4000–425 cm^{-1}. The ^1H NMR spectra were recorded on a JEOL FX 90Q/JEOL AL 300 MHz FT NMR spectrometer in DMSO-d_6/$CDCl_3$ using TMS as an internal reference. The ^{31}P NMR spectra were recorded on a JEOL AL 300 MHz FT NMR spectrometer at 121.49 MHz in DMSO-d_6/$CDCl_3$ using TMS and 85% H_3PO_4 as internal and external reference, respectively, at room temperature. FAB mass spectra were recorded on a JEOL SX 102/ DA-6000 mass spectrometer. Nitrogen was estimated by the Kjeldahl method, and sulfur was estimated by the Messenger method. Chlorine was estimated volumetrically by the Volhard method. Phosphorous was estimated as ammonium phosphomolybdate. The molecular weights were determined by the Rast camphor method.

TABLE 3 ^1H NMR and ^{31}P NMR Spectral Data (δ, ppm) of **1a** and Organophosphates (**2a–b, 3a–b**, and **4a–b**)

	^1H NMR		*^{31}P NMR*
Compound	–SH	Aromatic Proton	
1a	4.54 (bs)	6.8–7.6 (m)	
2a		7.0–7.9 (m)	62.5
2b		6.9–7.2 (m)	65.8
3a		7.1–7.6 (m)	64.6
3b		6.9–7.9(m)	68.5
4a		7.3–7.8 (m)	65.8
4b		6.9–8.0 (m)	76.5

bs = broad singlet; m = multiplets.

Synthesis of 2- Substituted Benzoxazole

2-(2′-Mercaptophenyl)benzoxazole (**1a**) was prepared by reacting the equimolar amounts of *o*-aminophenol and thiosalicylic acid in freshly prepared polyphosphoric acid. The reaction was carried out according to the procedure reported in the literature [21,22].

*Synthesis of $(C_{13}H_8NOS)P(O)Cl_2$ (**2a**) and $(C_{13}H_8NOS)P(S)Cl_2$ (**2b**)*

2-(2′-Mercaptophenyl)benzoxazole (**1a**) (0.001 mol) in dry THF (30 mL) and triethylamine (0.001 mol) in dry THF (20 mL) were taken in a flame-dried three-necked round-bottom flask, and temperature was kept at 0°C by keeping the flask in ice bath. To this mixture, a solution of $POCl_3$/$PSCl_3$ (0.001 mol) in dry THF (30 mL) was added dropwise at 0°C. After mixing the reactants, stirring was continued for 4 h at 0°C. Furthermore, the reaction was removed from the ice bath and refluxed under nitrogen atmosphere for 14–16 h with continuous stirring. Then it was cooled and filtered through a closed sintered

TABLE 4 Mass Spectral Data of **2a**

Compound	*m/z*(%)
2a	342.16 (10.53%), 306.66 (9.24%), 272.02 (9.40%), 225.1 (5.6%), 192.18 (14.6%), 115.14 (23.5%), 102 (89%), 77 (45%)

Relative abundance is given in parentheses.

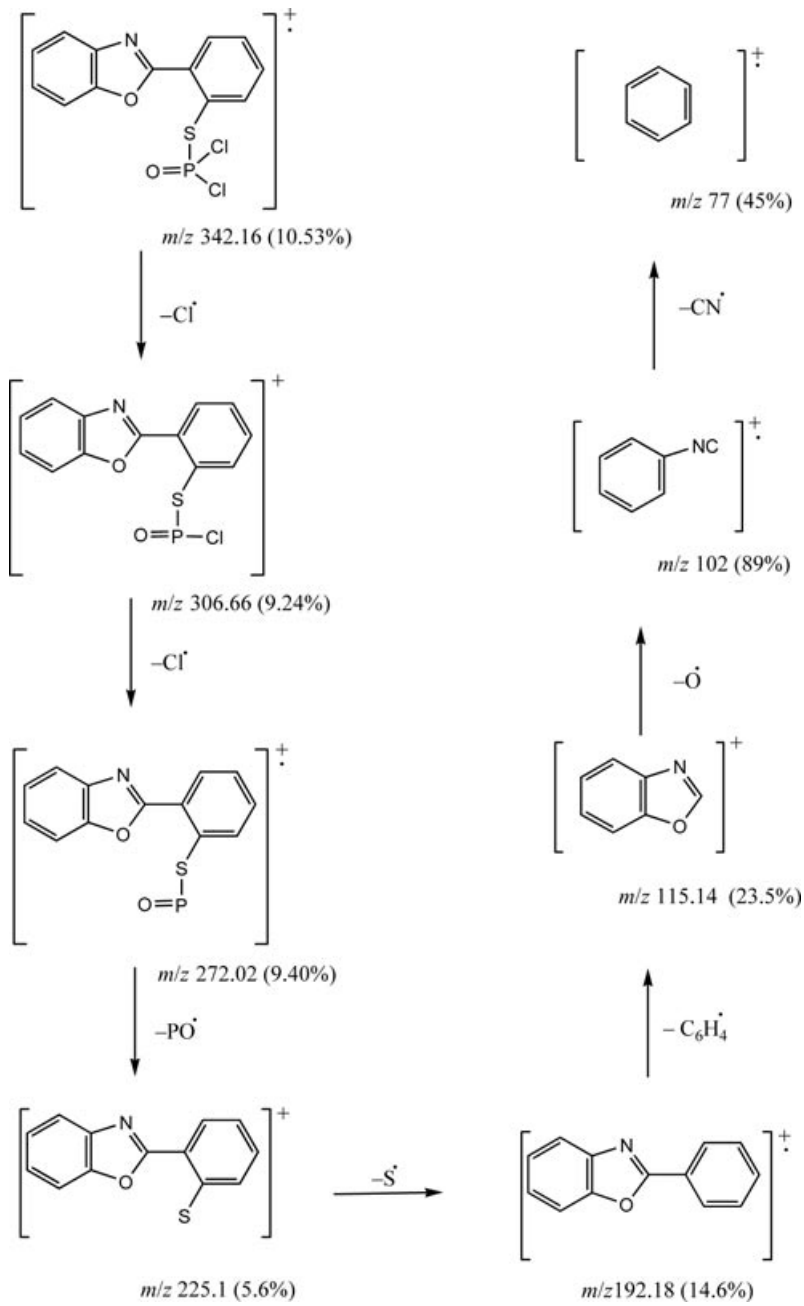

FIGURE 2

funnel to separate $Et_3N{\cdot}HCl$ formed during the reaction. The filtrate was concentrated under reduced pressure and was kept for crystallization in vacuum desiccators for 2 days. The product was recrystallized in ethanol and dried in vacuo.

*$(C_{13}H_8NOS)_2P(O)Cl$ (**3a**) and $(C_{13}H_8NOS)_2P(S)Cl$ (**3b**)*

2-(2′-Mercaptophenyl)benzoxazole (**1a**) (0.002 mol) in dry THF (30 mL) and triethylamine (0.002 mol) in dry THF (20 mL) were taken in a flame-dried three-necked round-bottom flask, and temperature was kept at 0°C by keeping the flask in ice bath. To this mixture, a solution of $POCl_3/PSCl_3$ (0.001 mol) in dry THF (30 mL) was added dropwise at 0°C. The reaction was carried out in a similar manner as described above.

*$(C_{13}H_8NOS)_3P(O)$ (**4a**) and $(C_{13}H_8NOS)_3P(S)$ (**4b**)*

2-(2′-Mercaptophenyl)benzoxazole (**1a**) (0.003 mol) in dry THF (30 mL) and triethylamine (0.003 mol)

TABLE 5 Antifungal Screening Data of **1a** and Organophosphates (**2a–b, 3a–b**, and **4a–b**)

	Inhibition after 72 h (%)					
	Aspergillus niger			*Fusarium oxysporium*		
Compound	50 (ppm)	100 (ppm)	200 (ppm)	50 (ppm)	100 (ppm)	200 (ppm)
1a	38.0	47	62	40	48	64
2a	58.2	64.66	78	60.12	63.33	74.26
2b	63.46	68.02	81.32	64.01	64	76
3a	60.14	64.98	80.06	66	69.01	78.39
3b	64.22	71	82.36	72.80	73.39	84.64
4a	64.78	69	78.35	66.73	78	92.06
4b	68.42	78.06	83	73	82.78	97.24
Bavistin	77	89	100	75	93	100

in dry THF (20 mL) were taken in a flame-dried three-necked round-bottom flask, and temperature was kept at 0°C by keeping the flask in ice bath. To this mixture, a solution of $POCl_3/PSCl_3$ (0.001 mol) in dry THF (30 mL) was added dropwise at 0°C. Then the reaction was carried out in a similar manner as describe above. The results of analytical and physical studies are summarized in Table 1.

BIOLOGICAL ACTIVITY

Antifungal Activity

The antifungal activity of **1a** and organophosphates (**2a–b, 3a–b**, and **4a–b**) was evaluated by the radial growth method using potato dextrose agar medium having the composition glucose 20 g, starch 20 agar–agar 20 g- and distilled water 1000 mL. To this medium, the requisite amount of the compound, after being dissolved in dimethylformamide (DMF) so as to obtain certain final concentrations (50, 100 and 200 ppm), was added. The organisms used in these investigations included *Aspergillus niger* and *Fusarium oxysporium*. The medium was then poured into the Petri dish/Petri plates and a small disc (0.7 cm) of the fungus culture was cut with a sterile cork borer and transferred aseptically to the center of a Petri dish, containing the medium with a certain amount of the compound (**1a, 2a–b, 3a–b**, and **4a–b**). Control setup without compounds was also maintained, and a standard fungicide (Bavistin) was also maintained. These Petri dishes were wrapped in polythene bags containing a few drops of dimethylformamide and were placed in an incubator at 25 ± 2°C. Three replicates were used in each case. The colony diameter, after 72 h, compared with control was taken as a measure of fungi toxicity. The percentage inhibition was calculated as $100(C–T)C^{-1}$, where C and T are the diameters of the fungus colony in the control and test plates, respectively (Table 5).

Insecticidal Activity

The insecticidal effect of **1a** and organophosphates (**2a–b, 3a–b**, and **4a–b**) has been evaluated on the mortality rate of *Periplenata americana* (adult

TABLE 6 Insecticidal Activity of **1a** and Organophosphates (**2a–b, 3a–b, 4a–b**)

	Mortality (%)								
	24 (h)			*48 (h)*			*72 (h)*		
Compound	20 (μg/cm^2)	40 (μg/cm^2)	60 (μg/cm^2)	20 (μg/cm^2)	40 (μg/cm^2)	60 (μg/cm^2)	20 (μg/cm^2)	40 (μg/cm^2)	60 (μg/cm^2)
1a	20	28	35	25	30	38	30	35	40
2a	55	78	85	76	87	92	78	88	92
2b	62	84	89	84	92	95	89	92	96
3a	58	80	86	78	92	93	80	92	94
3b	63	90	94	80	93	95	86	96	98
4a	60	82	88	77	91	95	82	90	95
4b	65	92	96	82	95	98	88	97	99
Malathion (standard)	80	95	99	88	99	100	90	99	100

cockroaches). The test was performed at room temperature in plastic box of $10 \times 10 \times 12$ cm^3 by contact and topical method. The effect of different concentrations (20, 40, and 60 μg/cm^2) of these compounds was evaluated as average percent mortality of *Periplenata americana* (adult cockroaches). The results are summarized in Table 6.

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REFERENCES

[1] Adele, F.; Davidson, G. M.; Raithby, Paul R.; Stalke, D.; Snaith, R. J Chem Soc, Dalton Trans 1995, 19, 3139.
[2] Preti, C.; Tosi, G. J Coord Chem 1978, 8, 15.
[3] Yalcin, I.; Oren, I.; Sener, E.; Akin, A.; Ucarturk, N. Farmaco 1998, 53, 337.
[4] Senser, E. A.; Arpaci-Temiz, O.; Yalcin, I.; Altanlar, N. Farmaco 2000, 55, 397.
[5] Sengupta, S. K.; Pandey, O. P.; Rao, G. P.; Dwivedi, A.; Singh, P. Phosphorus Sulfur Silicon 2003, 178, 839.
[6] Sengupta, S. K.; Pandey, O. P.; Rao, G. P.; Vishen, P. Metal Based Drugs 2000, 8, 293.
[7] Liu, Z.; Li, Z. Yuouji Huaxue 1991, 11(4), 433.
[8] Rose, U. J Heterocy Chem 1992, 29(2), 551.
[9] Sundberg, R. J.; Dalvie, D.; Cordero, J.; Sabat, M.; Musallam, H. A. Chem Res Toxicol 1993, 6(4), 500.
[10] Vyas, P. C.; Kaur, N.; Vyas, S. Heterocycl Commun 1997, 3, 91.
[11] Rida, Samia M.; Ashour, Fawzia A.; El-Hawash, Soad, A. M.; El-Semary, Mona M.; Badr, Mona H.; Shalaby, Monal A. Euro J Med Chem 2005, 40(9), 949.
[12] Kumar, D.; Jacob, M. R.; Reynolds, M. B.; Kerwin, S. M. Biorg Med Chem 2002, 10, 3997.
[13] Pareek, S.; Vyas, Sushma,; Seth, G.; Vyas, P. C. Heteroatom Chem 2008, 19, 154.
[14] Samota, M. K.; Kumar, A.; Kaur, J.; Mittal, S.; Seth, G. Phosphorus Sulfur Silicon 2006, 181, 1919.
[15] Mittal, S.; Samota, M. K.; Kaur, J.; Seth, G. Phosphorus Sulfur Silicon 2006, 181, 2843.
[16] Mittal, S.; Samota, M. K.; Kaur, J.; Seth, G. Phosphorus Sulfur Silicon 2007, 182, 2105.
[17] Moriguchi, T.; Okada, K.; Seio, K.; Sekine, M. Lett Org Chem 2004, 1, 140.
[18] Becke-Goehring, M.; Leichner, L.; Scharf, B. Z Anorg Allgem Chem 1966, 343, 154.
[19] Tebby, J. C.; Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis; Verkade, J. G.; Ouin, L. D. (Eds.); VCH:New York, 1987; Vol. 8, p. 1.
[20] Kabra, V.; Gupta, N.; Jain, S.; Saxena, V. Heteroatom Chem 2003, 14(6), 498.
[21] Hein, D. W.; Alhein, R. J.; Leawitt, J. J. J Am Chem Soc 1957, 79, 427.
[22] Mansingh, P. S.; Mohanty, R. R; Jena, S.; Dash, K. C. Indian J Chem A 1996, 35, 479.