Chem Sci Trans., 2013, **2(S1),** S167-S172
DOI:10.7598/cst2013.39

Chemical Science Transactions
ISSN/E-ISSN: 2278-3458/2278-3318

RESEARCH ARTICLE

One Pot Synthesis, Characterisation and Antimicrobial Activity of *α*- Amino Phosphonates†

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Received 5 February 2013 / Accepted 15 February 2013

Abstract: The synthesis of some new *α*-amino phosphonates (**4a-j**) was accomplished by one pot reaction of equimolar quantities of 2-amino-4-chloro-5-nitro phenol (**1**), aromatic aldehydes (**2a-j**) and diethyl / dimethylphosphite (**3**) in dry tetrahydrofuran at reflux condition via Kabachnik – fields reaction in high yields. All the synthesized compounds were characterised by IR, ^{1}H, ^{13}C, ^{31}P NMR and examined for their antimicrobial activity and found to possess good activity.

Keywords: *α*-Amino phosphonates, Kabachnik - fields reaction, Antimicrobial activity

Introduction

Synthesis of *α*-amino phosphonates exhibiting high bioactivity has recently attracted a lot of attention[1-3]. Applications include inhibition of synthase[4], HIV protease[5], renin[6] and PTPases[7,8]. Some of the derivatives of *α*-amino phosphonates are potential antibiotics[9] or herbicides[10], some are chief substrates in the synthesis of phosphoropeptides[11]. Among the number of synthetic approaches to *α*-amino phosphonates, one of the most powerful method is Kabachnik - fields reaction[12,13]. So we made an attempt to synthesise new series of *α*-amino phosphonates.

Experimental

Chemicals were purchased from Sigma-Aldrich. All of the products were identified by their physical and spectral data. The IR spectra were recorded as KBr pellets on Bruker. ^{1}H, ^{13}C, ^{31}P NMR were recorded on a Bruker AMX 500 MHz spectrometer operating at 500 MHz for ^{1}H, 125 MHz for ^{13}C and 202 MHz for ^{31}P NMR. All these compounds were dissolved in DMSO-d_6. The chemical shifts in δ were referenced to TMS (^{1}H and ^{13}C) and 85 % H_3PO_4 (^{31}P).

*General procedure for the synthesis of α-amino phosphonates (**4a-j**)*

To a stirred solution of 2-amino-4-chloro-5-nitro phenol (0.003 mol), the aldehyde (0.003 mol) in anhydrous tetrahydrofuran (20 mL) was added drop wise and the stirring continued at room temperature for 1 h. Then, dimethyl/ diethyl phosphite (0.003 mol) in dry

†Presented to the National Conference on Chemistry Solutions at SRM University, India

tetrahydrofuran (20 mL) was added drop wise. Stirring was continued at room temperature for another 0.5 h and the mixture was heated at gentle reflux for 5-6 h. The progress of the reaction was monitored by TLC analysis. After completion of the reaction the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (80-120 mesh) using ethyl acetate: hexane (2:1) as eluent.

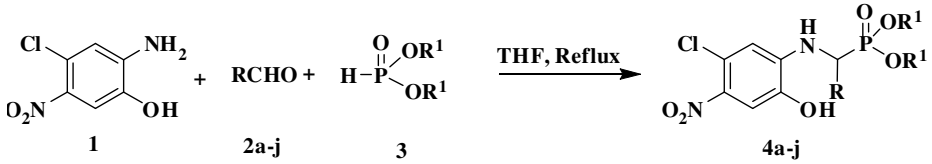
Antimicrobial activity

The antimicrobial activities of the test compounds were evaluated (Table 1 & 2) and their effect was compared to the stranded antibiotic penicillin and anti-fungal agent griseofulvin. Compounds (**4a-j**) were screened for their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* by the disc diffusion method[18,19] in nutrient agar medium at various concentrations (100, 250 µg/disc) in dimethyl formamide (DMF). These solutions were added to each filter disc and DMF was used as control. The plates were incubated at 35 ˚C and examined for zone of inhibition around each filter disc after 24 h. Their anti-fungal activity was evaluated against *Aspergillus niger* and *Fusarium oxysporium* of 100 and 250 µg/disc. Fungal cultures were grown on potato dextrose broth at 25 ˚C and finally spore suspension was adjusted to 10^5 spores/mL. Most of the compounds showed significant activity against bacteria and low activity against fungi.

Results and Discussion

α-Amino phosphonates (**4a-j**) were synthesized by three component one-pot reaction of equimolar quantities of 2-amino-4-chloro-5-nitro phenol (**1**), dimethyl/ diethyl phosphite (**2**) and various aldehydes (**3a-j**) in dry tetrahydrofuran at reflux conditions for 5-6 h (Scheme 1) . Progress of the reaction was monitored by TLC analysis and products were purified by column chromatography using ethyl acetate:hexane (2:1) as eluent. The chemical structures of all the new compounds were confirmed by elemental analysis, IR ^{1}H, ^{13}C and ^{31}P NMR.

Compounds **4a-j** exhibited characteristic IR stretching frequencies in the region[14] 742-769, 1161-1264 and 3314-3404 cm^{-1} for P-C$_{(aliphatic)}$, P=O and N-H respectively. The aromatic protons of the two benzene rings of the compounds 4a-j showed a mutiplet at δ 6.42-7.54. The P-C-H proton signal appeared as multiplet[15] at δ 3.92-4.24 due to its coupling with phosphorus and proton of N-H. The N-H proton signal appeared as singlet. The methylene protons of P-OC<u>H$_2$</u>CH$_3$ showed a multiplet and methyl protons of P-OCH$_2$ C<u>H$_3$</u> showed a triplet in the region of δ 3.55-3.99 and δ 1.05-1.38 respectively[16]. ^{31}P NMR signals[17] appeared in the region 22.59-27.86 ppm for all the compounds **4a-j**.



1 **2a-j** **3** **4a-j**

Scheme 1

Compd	R	R^1	Compd	R	R^1
2a & 4a	4-OHC$_6$H$_4$	C$_2$H$_5$	2f & 4f	4-OHC$_6$H$_4$	CH$_3$
2b & 4b	3,4-(OMe)$_2$C$_6$H$_3$	C$_2$H$_5$	2g & 4g	3,4-(OMe)$_2$C$_6$H$_3$	CH$_3$
2c & 4c	4-ClC$_6$H$_4$	C$_2$H$_5$	2h & 4h	4-ClC$_6$H$_4$	CH$_3$
2d & 4d	2-NO$_2$C$_6$H$_4$	C$_2$H$_5$	2i & 4i	2-NO$_2$C$_6$H$_4$	CH$_3$
2e & 4e	4-OMeC$_6$H$_4$	C$_2$H$_5$	2j & 4j	4-OMeC$_6$H$_4$	CH$_3$

Table. 1. Antibacterial activity of **4a-j**

Compound	Zone of inhibition, mm			
	Escherichia coli		*Staphylococcus aureus*	
	100[a] µg/disc	250[a] µg/disc	100[a] µg/disc	250[a] µg/disc
4a	9	16	8	13
4b	7	11	8	14
4c	8	12	6	11
4d	6	10	-	9
4e	-	10	7	12
4f	8	14	6	12
4g	6	13	5	9
4h	9	16	9	15
4i	5	9	6	10
4j	-	7	-	-
Penicillin[b]		20		20

[a]*Concentration in ppm,* [b]*standard reference*

Table 2. Antifungal activity of **4a-j**

Compound	Zone of inhibition, mm			
	Aspergillus niger		*Fusarium oxysporium*	
	100[a] µg/disc	250[a] µg/disc	100[a] µg/disc	250[a] µg/disc
4a	4	7	3	7
4b	5	9	5	8
4c	-	5	3	5
4d	7	9	-	5
4e	5	8	4	7
4f	6	10	6	8
4g	-	4	-	4
4h	-	6	4	7
4i	5	8	5	8
4j	3	7	2	-
Griseofulvin[b]		21		20

[a]*Concentrationin ppm,* [b]*standard reference*

Physical, analytical, IR and NMR data of compounds 3a-j

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(4-hydroxyphenyl)methyl] diethyl-phosphonate (**4a**)*

Yield 79%; m.p. : 172-174 °C. IR: 765 (P-$C_{(aliphatic)}$), 1215 (P=O), 3335 (N-H) cm^{-1}. ^{1}H NMR : δ 1.15 (t, 3H, P-$OCH_2C\underline{H_3}$), 1.26 (t, 3H, P-$OCH_2C\underline{H_3}$), 3.69-3.72 (m, 2H, P-$OC\underline{H_2}$ CH_3), 3.91-3.95 (m, 2H, P-$OC\underline{H_2}CH_3$), 4.13-4.18 (m, 1H, P-CH), 5.41 (s, 1H, N-H), 6.72-7.54 (m, 6H, Ar-H), 10.3 (s, 1H, Ar-OH). ^{13}C NMR: δ 16.7, 55.8, 56.1, 111.2, 113.2, 120.5, 132.5, 141.8, 145.0. ^{31}P NMR: δ 22.60. Anal.Calcd for $C_{17}H_{20}ClN_2O_7P$: C, 47.38; H, 4.64; N, 6.50. Found: C, 47.29; H, 4.61; N, 6.45.

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(3,4-dimethoxyphenyl)methyl] diethyl-phosphonate (**4b**)*

Yield 65% ; m.p. : 189-191 ˚C. IR: 756 (P-$C_{(aliphatic)}$), 1196 (P=O), 3356 (N-H) cm^{-1}.^{1}H NMR : δ 1.25 (t, 3H, P-$OCH_2C\underline{H}_3$), 1.33 (t, 3H, P-$OCH_2C\underline{H}_3$), 3.78-3.82 (m, 2H, P-$OC\underline{H}_2$ CH_3), 3.95-3.98 (m, 2H, P-$OC\underline{H}_2CH_3$), 4.19-4.24(m, 1H, P-CH), 5.1 (s, 1H, N-H), 6.52-7.34 (m, 5H, Ar-H), 10.1 (s, 1H, Ar-OH). ^{13}C NMR: δ 15.4, 53.1, 56.9, 65.0, 108.4.2, 115.2, 119.5, 134.5, 139.2, 141.4. ^{31}P NMR: δ 26.67. Anal.Calcd for $C_{19}H_{24}ClN_2O_8P$: C, 48.05 ; H, 5.05 ; N, 5.90. Found: C, 48.01; H, 5.02; N, 5.85.

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(4-chorophenyl)methyll]diethyl phosphor-nate (**4c**)*

Yield:72% ; m.p.: 161-163 ˚C. IR: 769 (P-$C_{(aliphatic)}$), 1189 (P=O), 3346 (N-H) cm^{-1}. ^{1}H NMR : δ 1.05 (t, 3H, P-$OCH_2C\underline{H}_3$), 1.32 (t, 3H, P-$OCH_2C\underline{H}_3$), 3.59-3.63 (m, 2H, P-$OC\underline{H}_2$ CH_3), 3.82-3.85 (m, 2H, P-$OC\underline{H}_2CH_3$), 4.09-4.13 (m, 1H, P-CH), 5.23 (s, 1H, N-H), 6.62-7.52 (m, 6H, Ar-H), 10.23 (s, 1H, Ar-OH). ^{31}P NMR: δ 22.59. Anal.Calcd for $C_{17}H_{19}Cl_2N_2O_6P$: C, 45.43; H, 4.23; N, 6.23. Found: C, 45.32; H, 4.18; N, 6.19.

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(2-nitrophenyl)methyl]diethylphosphor-nate (**4d**)*

Yield: 64% ; m.p. : 165-167 ˚C. IR : 747 (P-$C_{(aliphatic)}$), 1161 (P=O), 3399 (N-H) cm^{-1}. ^{1}H NMR : δ 1.22 (t, 3H, P-$OCH_2C\underline{H}_3$), 1.32 (t, 3H, P-$OCH_2C\underline{H}_3$), 3.65-3.69 (m, 2H, P-$OC\underline{H}_2$ CH_3), 3.77-3.81 (m, 2H, P-$OC\underline{H}_2CH_3$), 4.11-4.15 (m, 1H, P-CH), 5.22 (s, 1H, N-H), 6.52-7.51 (m, 6H, Ar-H), 10.11 (s, 1H, Ar-OH). ^{31}P NMR: δ 24.12. Anal.Calcd for $C_{17}H_{19}ClN_3O_8P$: C, 44.39; H, 4.13; N, 9.14. Found: C, 44.32; H, 4.08; N, 9.09.

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(4-methoxyphenyl)methyl] diethylphospho-nate (**4e**)*

Yield 76%; m.p.: 182-184 ˚C. IR : 752 (P-$C_{(aliphatic)}$), 1196 (P=O), 3348 (N-H) cm^{-1}. ^{1}H NMR : δ 1.15 (t, 3H, P-$OCH_2C\underline{H}_3$), 1.28 (t, 3H, P-$OCH_2C\underline{H}_3$), 3.53-3.57 (m, 2H, P-$OC\underline{H}_2$ CH_3), 3.75-81 (m, 2H, P-$OC\underline{H}_2CH_3$), 4.15-21 (m, 1H, P-CH), 5.14 (s, 1H, N-H), 6.42-7.24 (m, 6H, Ar-H), 10.24 (s, 1H, Ar-OH). ^{31}P NMR: δ 25.67. Anal.Calcd for $C_{18}H_{22}ClN_2O_7P$: C, 48.59; H, 4.94; N, 6.29. Found: C, 48.52; H, 4.89; N, 6.23.

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(4-hydroxyphenyl)methyl]dimethyl-phospho-nate (**4f**)*

Yield 66%; m.p. : 186-188 ˚C.IR: 745 (P-$C_{(aliphatic)}$), 1216 (P=O), 3382 (N-H) cm^{-1}. ^{1}H NMR: δ 3.43 (d, 3H, P-O-$C\underline{H}_3$), 3,52 (d, 3H, P-O-$C\underline{H}_3$), 4.01-4.06 (m, 1H, P-CH), 5.1 (s, 1H, N-H), 6.56-7.44 (m, 6H, Ar-H), 10.2 (s, 1H, Ar-OH). ^{31}P NMR: δ 25.08. Anal.Calcd for $C_{15}H_{16}ClN_2O_7P$: C, 44.72; H, 3.97; N, 6.95. Found: C, 44.66; H, 3.92; N, 6.91.

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(3,4-dimethoxyphenyl)methyl] dimethyl-phosphonate (**4g**)*

Yield 74%; m.p. : 146-148 ˚C. IR: 751 (P-$C_{(aliphatic)}$), 1247 (P=O), 3404 (N-H) cm^{-1}. ^{1}H NMR: δ 3.61 (d, 3H, P-O-$C\underline{H}_3$), 3.72 (d, 3H, P-O-$C\underline{H}_3$), 3.95-4.01 (m, 1H, P-CH), 4.92 (s, 1H, N-H), 6.55-7.34 (m, 5H, Ar-OH), 10.26 (s, 1H, Ar-H). ^{31}P NMR: δ 27.23. Anal.Calcd for $C_{17}H_{20}ClN_2O_8P$: C, 45.68; H, 4.47; N, 6.27. Found: C, 45.61; H, 4.42; N, 6.23.

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(4-chorophenyl)methyl] dimethylphosphonate (**4h**)*

Yield 63% ; m.p. : 195-197 ˚C. IR: 758 (P-$C_{(aliphatic)}$),1198 (P=O),3372 (N-H) cm^{-1}. ^1H NMR: δ 3.26 (d, 3H, P-O-C$\underline{H}_3$), 3.45 (d, 3H, P-O-C$\underline{H}_3$), 3.90-3.96 (m, 1H, P-CH), 5.14 (s, 1H, N-H), 6.70-7.48 (m, 6H, Ar-H), 10.13 (s, 1H, Ar-OH). ^{31}P NMR: δ 27.36. Anal.Calcd for $C_{15}H_{15}Cl_2N_2O_6P$: C, 42.75; H, 3.56; N, 6.65. Found: C, 42.68; H, 3.52; N, 6.59.

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(2-nitrophenyl)methyl]dimethylphosphonate (**4i**)*

Yield: 70%; m.p. : 178-180˚C. IR: 751(P-$C_{(aliphatic)}$), 1264 (P=O), 3359(N-H) cm^{-1}. ^1H NMR: δ 3.33 (d, 3H, P-O-C$\underline{H}_3$), 3.51 (d, 3H, P-O-C$\underline{H}_3$),4.13-4.17 (m, 1H, P-CH), 5.11 (s, 1H, N-H), 6.45-7.54 (m, 6H, Ar-H), 10.2 (s, 1H, Ar-OH). ^{31}P NMR: δ 26.78. Anal.Calcd for $C_{15}H_{15}ClN_3O_8P$: C, 41.71; H, 3.47; N, 9.73. Found: C, 41.64; H, 3.40; N, 9.68.

*[(5-Chloro-2-hydroxy-4-nitrophenylamino)-(4-methoxyphenyl)methyl]dimethylphosphonate (**4j**)*

Yield 76% ; m.p. : 156-158 ˚C. IR: 743(P-$C_{(aliphatic)}$), 1191(P=O), 3314 (N-H) cm^{-1}. ^1H NMR: δ 3.65 (d, 3H, P-O-C$\underline{H}_3$), 3.79 (d, 3H, P-O-C$\underline{H}_3$),4.17-4.22(m, 1H, P-CH), 5.14 (s, 1H, N-H), 6.62-7.34 (m, 6H, Ar-H), 10.05 (s, 1H, Ar-OH). ^{31}P NMR : δ 27.86. Anal.Calcd for $C_{16}H_{18}ClN_2O_7P$: C, 46.09; H, 4.32; N, 6.72. Found: C, 46.01; H, 4.27; N, 6.67.

Conclusion

We have synthesized new *α*-amino phosphonates **4a-j** in high yields by Kabachnik – fields reaction without using any catalyst. All of them showed significant activity against bacteria and low activity against fungi.

Acknowledgement

The authors express thanks to ISISM, SRM University for providing spectral data and Faridha Begham for her help in the Antimicrobial assay.

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