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Original article

Synthesis of 2-aziridinyl phosphonates by modified Gabriel–Cromwell reaction and their antibacterial activities

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ABSTRACT

A set of new aziridinyl phosphonates (**4a**–**g**) were synthesized by using the Gabriel–Cromwell reaction and its modified version developed in this study and their structures confirmed by HRMS, IR, and NMR spectra. All the compounds were screened for their antibacterial activity. They all showed comparable moderate to good growth inhibitory activity in reference to ampicillin and streptomycin.

1. Introduction

Aziridines are important small molecules that are found in the structure of biologically active natural compounds and have many applications in organic chemistry [1]. They are used as precursors to amino acids, azomethine ylides, and amino alcohols. In addition they are used as monomers for polymerization [2], chiral auxiliaries [3], and chiral ligands [4]. Aziridinyl phosphonates are the precursors of α-amino phosphonates that find applications such as enzyme inhibitors [5], as haptens for catalytic antibodies [6], as antibacterial agents [7], and herbicides [8]. Although many studies have been carried out for the synthesis of aziridine-2-carboxylate esters, there are only limited number of studies reported for the synthesis of aziridinyl phosphonates [9]. One of the methods reported by Kim et al. involves the nitrene addition to cinnamoyl phosphonate derivatives which produced N-tosyl-2-aziridinyl phosphonates in 81–89% yield [10]. Another synthesis reported by Stevens et al. gives rather specific N-vinylaziridinyl phosphonate derivatives in 29–57% yield [11]. Davis et al. reported the synthesis of chiral aziridinyl phosphonates in 76% yield by reacting chiral sulfinimines with chloromethyl phosphonates [12]. Loreto et al. also studied synthesis of aziridinyl phosphonates by (ethoxycarbonyl)nitrene addition to α,β-unsaturated phosphonates which yielded aziridines in 14–45% [13]. In the study of Lesniak et al. 1,3-dipolar cycloaddition of diazophosphonate with aryl imines produced aziridinyl phosphonates in 67–78% yield after a period of 15 days [14]. Very recently, Hodgson et al. reported aziridinyl phosphonate synthesis by lithiation-induced phosphonyl migration from nitrogen to carbon in terminal aziridines [15]. Due to the limited number of studies and also the limitations in the application of some of the methods such as low yields, long reaction times, or specific aziridine synthesis there is a need for the development of more general methods for the synthesis of aziridinyl phosphonates. Our group has been involved in the synthesis of new aziridine derivatives to use them as chiral ligands for metal catalyzed enantioselective synthesis of organic compounds [16]. As an extention of previous studies, here in we report the synthesis of new aziridinyl phosphonates by modified Gabriel–Cromwell reaction and also their antibacterial activities.

2. Results and discussion

2.1. Chemistry

One of the commonly used methods for the aziridine synthesis is the Gabriel–Cromwell reaction Scheme 1. A nice asymmetric application of this method was reported by Garner et al. [17]. Berlin et al. applied this method for the synthesis of diethyl 2-aziridinyl phosphonate (**4**, R = H) which was obtained in 62% yield by heating dibromo compound **2** with ammonia in a sealed tube Scheme 1 [18]. This was the only aziridine reported by this group using the

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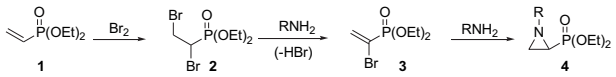
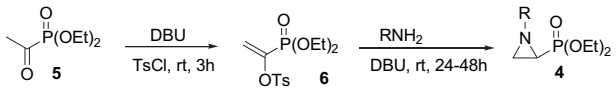


Scheme 1. Berlin's synthesis of aziridinyl phosphonate by Gabriel–Cromwell reaction.

Gabriel–Cromwell reaction. The steps of this reaction involves addition of Br_2 to vinyl group to form compound **2**. Then HBr elimination by triethylamine or the amine used for aziridination gives α-bromo phosphonate **3**. Michael addition of another equivalent of amine to **3** then S_N2 displacement of bromide leads to the aziridine **4**.

However, the vinyl phosphonate **1** is difficult to synthesize [19] and highly expensive. As an alternative to this method we planned to start with acetyl phosphonate which can be synthesized easily using the Michaelis–Arbusow reaction [20]. After the synthesis of acetyl phosphonate **5**, the next step was the conversion of this compound to tosylate **6** Scheme 2. Since it was necessary to have a good leaving group at α-position of the phosphonate. This conversion was made possible by treatment of acetyl phosphonate with DBU and tosyl chloride.

In order to synthesize compound **6** in high yield, it was necessary to optimize the reaction conditions. For this reason, different solvents (THF, DCM, CH_3CN, MeOH, and $CHCl_3$) were tried. Among the solvents, $CHCl_3$ and DCM gave similar results, and tosylate **6** was obtained in about 50% yield. Product formation was not observed in methanol and the highest yield (87%) was obtained with acetonitrile. In the case of THF, yield was 3–5% lower compared to acetonitrile. We have tried two bases DBU and Et_3N but almost no product formation took place in Et_3N. Therefore, DBU was chosen as the base. After determining solvent and base, different reaction times were tried. Increasing the reaction time from 3 h to 24 h didn't change the yield considerably. Change of reaction concentration had a significant effect on the yield. The highest yield was obtained when the concentration was about 0.2 M. Reactions carried out at 0.5 M and 1.0 M gave the product in low yield. We have also tried to synthesize mesylate instead of tosylate but the yield was low. After determining the optimum conditions for the synthesis of tosylate **6**, the aziridine forming step was carried out Scheme 2. Again, after doing some optimization studies by using different solvents (THF, DCM, $CHCl_3$, and CH_3CN), different amine concentrations and reaction times, it was found that the highest yield could be obtained by stirring tosylate **6** with amine (1.4 equiv) and DBU (1.0 equiv) at rt for 24 h in CH_3CN. After optimizing the reaction conditions, different amines were used to test the applicability of this method. The same aziridines were also synthesized by the Gabriel–Cromwell reaction (Method B, Scheme 1) in order to compare the efficiency of two methods. The results of both methods are summarized in Table 1. The absolute stereochemistry at the aziridine chirality center of **4e**, **4e'**, **4f**, and **4f'** were not determined.



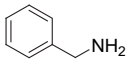
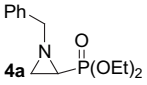
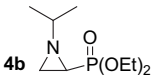
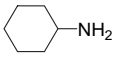
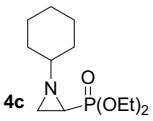
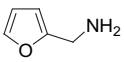
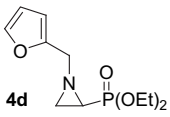
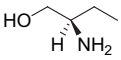
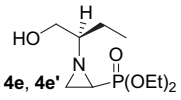
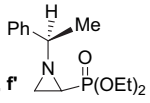
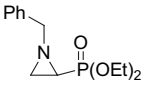
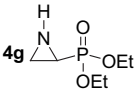
Scheme 2. Synthesis of aziridinyl phosphonate from acetyl phosphonate.

2.2. Antibacterial studies

The newly synthesized aziridinyl phosphonates **4a**–**g** were screened for their antibacterial activity against *Bacillus subtilis* (Gram-positive), *Escherichia coli* DH5α (Gram-negative), and three fresh water isolates, namely Fs48 (*Gordonia* spp) (Gram-positive to variable), Fs30 (*Brevundimonas* spp) (Gram-negative), and Fs24 (*Kocuria* spp) (Gram-positive) by performing disc diffusion assays [21]. The Fs48, 30, and 24 were isolated from fresh water fish surface mucus and identified at genus level with 16s ribosomal DNA sequencing. Ampicillin and Streptomycin were used as the reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zone of bacterial growth around the disks in mm (Table 2).

Results of antibacterial screening studies revealed that all the aziridinyl phosphonates showed moderate to good activity as compared to reference antibiotics. As can be seen from Table 2 aziridinyl phosphonate **4b** having benzyl group on the nitrogen showed better activity than reference antibiotics against *B. subtilis*. Aziridine **4e'** showed similar activity as amphicillin but better activity than Streptomycin against the same bacteria. In the case of *E. coli*, again, **4e'** showed the highest activity. Compounds **4b**, **4c**, **4d**, **4e'**, and **4f** showed similar activity against Fs48, **4c** showed the highest activity against this bacteria. For Fs30, all the compounds showed better activity than ampicillin and **4e** showed the highest inhibitory activity against this bacteria among the series of aziridinyl phosphonates. The compound **4e** showed the highest activity against Fs24 and the others **4a**, **4b**, **4c**, **4e'**, and **4f'** showed moderate inhibitory activity against the same bacteria.

Table 1
Synthesis of 2-aziridinyl phosphonates.

Entry	Amine	Product	Yield (%) Method A[a]	Yield (%) Method B[b]
1		 **4a**	88 (86)	85
2	$(CH_3)_2CHNH_2$	 **4b**	88 (62)	79
3		 **4c**	89 (76)	96
4		 **4d**	50 (92)	73
5		 **4e**, **4e'**	65 (78)	91
6		 **4f**, **f'**	76 (82)	71
7		 **4g**	85	

[a] Method A: Tosylate **6**, amine (1.4 equiv), and DBU (1 equiv) were stirred at rt for 24 h. In parenthesis, values obtained using acetonitrile as the solvent after 48 h stirring at rt.
[b] Method B: Dibromide **2** and amine (3 equiv) were refluxed in acetonitrile for 3 h.

Table 2
Antibacterial activities of aziridinyl phosphonates.

Compounds	Inhibition zone diameters (mm)				
	Bacillus subtilis	*Escherichia coli*	Fs48 *Gordonia spp*	Fs30 *Brevundimonas spp*	Fs24 *Kocuria spp*
4a	10 ± 0	8 ± 0	13 ± 0	13.66 ± 1.15	18 ± 1.73
4b	19.33 ± 1.15	9 ± 0	15.66 ± 3.78	16 ± 3.60	19 ± 2.64
4c	9.66 ± 0.57	10 ± 0	23.33 ± 2.88	14.33 ± 0.57	18.33 ± 2.88
4d	10.33 ± 0.57	13.33 ± 0.88	14.66 ± 0.57	15 ± 0	14.66 ± 0.57
4e	11 ± 2	10.66 ± 1.15	11 ± 1.41	20 ± 0	25 ± 0
4e'	17 ± 0	20 ± 0	15.33 ± 4.61	13 ± 3.46	18.66 ± 1.51
4f	10 ± 0	13 ± 1	13.33 ± 1.15	13.66 ± 1.15	12 ± 2
4f'	11.66 ± 1.15	12 ± 0	16.33 ± 1.51	13.33 ± 0.57	19 ± 1
4g	11.33 ± 2.30	11.33 ± 2.30	11.66 ± 2.88	12.33 ± 2.51	12.33 ± 2.51
Ampicillin	17.33 ± 2.3	16.67 ± 1.52	ND	10.67 ± 0.57	47.67 ± 0.57
Streptomycin	14 ± 0.0	19.67 ± 0.57	29 ± 1.73	29.33 ± 0.57	26 ± 6.9

It is important to note that diastereomeric aziridines **4e** and **4e'** behaved differently. While the first one showed highest activity (highest of all) against Fs24 *Kocuria spp*, second one showed the highest activity against *E. coli*. Also the diasteromers **4f** and **4f'** showed parallel activities against the first four bacteria in Table 2 but different activities against Fs24 *Kocuria spp*. Aziridine **4g** having no substituent on the nitrogen showed a low but very similar activity against all the bacteria. From these results it can be concluded that the substituent on the nitrogen as well as the stereochemistry of aziridine ring affects the antibacterial activity of these compounds.

3. Conclusion

A new method which can be considered as a modified Gabriel–Cromwell reaction was developed for the synthesis of 2-aziridinyl phosphonates. Using this method eight new aziridines including stereoisomers were synthesized in 70–92% yield. The same aziridines were also synthesized by using classical Gabriel–Cromwell reaction in 71–96% yield to compare the efficiency of two methods. Basically both methods form the aziridinyl phosphonates in similar yields. The main advantages of the method developed in this study are the ease of availability of the starting material **6** and the fact that the aziridination reaction proceeds at rt. The only disadvantage is the longer reaction times (24–48 h).

The antibacterial activities of synthesized aziridinyl phosphonates were tested for the first time in this study by performing disc diffusion assays. These studies showed that aziridine derivatives have different activities against different bacteria. The substituent on the nitrogen has a significant effect on the activity. The stereochemistry is also important, diasteromers **4e**, **4e'** and **4f**, **4f'** varied in their activity against different bacteria. Especially for the diastereomers **4e** and **4e'**, there is a significant difference in terms of antibacterial activity.

4. Experimental

4.1. Chemistry

IR spectra were recorded on FT-IR Shimadzu 8300 spectrophotometer. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker 400 MHz spectrometer in $CDCl_3$–CCl_4 using tetramethylsilane as internal standard and chemical shifts are reported in δ units. HRMS were recorded on a Waters Micromass Q-TOF instrument in ES^+ mode. Thin layer chromatography was performed on pre-coated silica plates (Merck Kiesegel 60 F254) and column chromatography using silica gel (mesh 230–400). Phosphomolybdic acid in ethanol and ninhydrin was used as visualizing agents.

4.1.1. Synthesis of 1-(diethoxyphosphoryl) vinyl 4-methylbenzenesulfonate (**6**)

To a dry two necked round bottom flask with a magnetic stir bar under N_2 atmosphere, diethyl acetyl phosphonate (100 μL, 0.62 mmol) and 4-methylbenzene-1-sulfonyl chloride (176 mg, 0.923 mmol) in CH_3CN (2.7 mL) was added. The reaction flask was cooled to 0 °C in an ice-water bath. Then, DBU (141 μL, 0.92 mmol) was slowly added. The resulting mixture was stirred at rt for 3 h. At the end of this time, water (10 mL) and CH_2Cl_2 (10 mL) was added to the reaction flsk. Two layers were separated and aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by flash column chromatography on silica gel (EtOAc, $R_f = 0.69$) to give **6** in 87% yield (179 mg, 0.536 mmol) as a light yellow solid. ^{1}H NMR δ 7.77 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 5.89 (ddd, J = 12.8, 11.8, 2.6 Hz, 2H), 4.10–3.84 (m, 4H), 2.41 (s, 3H), 1.24 (t, J = 7.1 Hz, 6H). ^{13}C NMR δ 147.21 (Ar), 144.95 (Ar), 133.43 (CH_2CP), 129.64 (Ar), 128.44 (Ar), 118.98 (d, J_{P-C} = 23.16 Hz), 62.86 (CH_2CH_3), 62.81 (CH_2CH_3), 21.64 (CH_3Ar), 16.18 (CH_3CH_2), 16.12 (CH_3CH_2). ^{31}P NMR δ 5.24.

4.2. General procedure for the synthesis of aziridinyl phosphonates **4** (Method A)

To a stirred mixture of 1-(diethoxyphosphoryl)vinyl 4-methylbenzenesulfonate (**6**, 0.83 mmol) and DBU (0.83 mmol, 1.0 equiv) was added amine (1.16 mmol, 2 equiv) at rt ad stirred for 24 h. When the reactions were carried out in CH_3CN, the concentration was adjusted to ~2 M with respect to amine. The resulting mixture was stirred at this temperature for 48 h. The crude product was purified by column chromatography using silica gel and hexane:ethyl acetat (1:1 mixture) as the eluent.

4.2.1. Diethyl 1-benzylaziridin-2-ylphosphonate (**4a**)

Colorless oil, (EtOAc, $R_f = 0.1$), yield 88% (197 mg, 0.732 mmol); ^{1}H NMR δ 7.40–7.07 (m, 5H), 4.14–3.71 (m, 4H), 3.55 (d, J = 13.0 Hz, 1H), 3.24 (d, J = 13.0 Hz, 1H), 2.12 (dd, J = 9.2, 3.5 Hz, 1H), 1.77–1.39 (m, 2H), 1.20 (t, J = 7.0 Hz, 3H), 1.13 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 137.78 (CH), 128.38 (CH), 128.28 (CH), 127.34 (CH), 65.27 (d, J = 7.3 Hz, CH_2Ph), 62.29 (d, J = 6.2 Hz, CH_2CH_3), 61.96 (d, J = 6.1 Hz, CH_2CH_3), 31.92 (d, J = 5.3 Hz, CH_2N), 31.71 (d, J_{P-C} = 216.9 Hz, PCH), 16.39 (CH_3), 16.33 (CH_3). ^{31}P NMR δ 22.48. IR (neat, cm^{-1}) 3062, 2981, 2930, 2906, 1454, 1019, 766. HRMS-EI (m/z): calcd for $C_{13}H_{21}NO_3P$ ($M + H^+$): 270.1259; found: 270.1254.

4.2.2. Diethyl 1-isopropylaziridin-2-ylphosphonate (**4b**)

Colorless oil, (EtOAc, R_f = 0.14), yield 88.4% (165 mg, 0.746 mmol); ^{1}H NMR δ 4.17–3.94 (m, 4H), 2.00 (ddd, J = 8.9, 3.5, 1.0 Hz, 1H), 1.45 (td, J = 7.1, 1.1 Hz, 1H), 1.41–1.32 (m, 2H), 1.27 (t,

J = 7.1 Hz, 6H), 1.10 (d, J = 8.9 Hz, 3H), 1.08 (d, J = 8.9 Hz, 3H). ^{13}C NMR δ 62.36 (d, J = 7.1 Hz, $CH(CH_3)_2$), 62.18 (d, J = 6.4 Hz, CH_2CH_3), 61.67 (d, J = 6.3 Hz, CH_2CH_3), 31.36 (d, J_{P-C} = 219.3 Hz, PCH), 31.07 (d, J = 5.2 Hz, CH_2N), 21.87 (C), 16.36 (CH_3), 16.30 (CH_3), 16.23 (CH_3). ^{31}P NMR δ 23.11. IR(neat, cm^{-1}) 3345, 2970, 2931, 2874, 1370, 1234, 1024, 970. HRMS-EI (m/z): calcd for $C_9H_{21}NO_3P$ (M + H$^+$): 222.1259; found: 222.1251.

*4.2.3. Diethyl 1-cyclohexylaziridin-2-ylphosphonate (**4c**)*

Colorless oil, (EtOAc, R_f = 0.29), yield 89% (210 mg, 0.804 mmol); ^{1}H NMR δ 4.15–3.99 (m, 4H), 2.04–1.95 (m, 1H), 1.73 (m, 4H), 1.58–1.31 (m, 5H), 1.27 (t, J = 7.0 Hz, 3H), 1.26 (t, J = 7.0 Hz, 3H), 1.21–0.97 (m, 4H). ^{13}C NMR δ 70.08 (d, J = 6.7 Hz, CHNCHP), 62.29 (d, J = 6.3 Hz, CH_2CH_3), 61.82 (d, J = 6.3 Hz, CH_2CH_3), 32.41 (CH_2CHCH_2), 30.78 (d, J_{P-C} = 219.2 Hz, PCH), 30.59 (d, J = 5.2 Hz, CH_2NCH), 25.93 (CH_2CH_2CH), 24.46 ($CH_2CH_2CH_2CH$), 16.45 (d, J = 6.1 Hz, CH_3CH_2), 16.36 (d, J = 6.2 Hz, CH_3CH_2). ^{31}P NMR δ 23.36. IR(neat, cm^{-1}) 2980, 2927, 2854, 1449, 1245, 1022, 961. HRMS-EI (m/z): calcd for $C_{12}H_{25}NO_3P$ (M + H$^+$): 262.1572; found: 262.1569.

*4.2.4. Diethyl 1-(furan-2-ylmethyl)aziridin-2-ylphosphonate (**4d**)*

Colorless oil, (EtOAc, R_f = 0.24), yield 50% (108 mg, 0.415 mmol); ^{1}H NMR δ 7.29 (dd, J = 1.8, 0.8 Hz, 1H), 6.25 (dd, J = 3.2, 1.8 Hz, 1H), 6.21 (d, J = 3.2 Hz, 1H), 4.07–3.92 (m, 4H), 3.62 (d, J = 13.9 Hz, 1H), 3.29 (d, J = 13.9 Hz, 1H), 2.09 (ddd, J = 9.2, 3.2, 1.2 Hz, 1H), 1.70–1.56 (m, 2H), 1.24 (t, J = 7.1 Hz, 3H), 1.19 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 151.22 (OCCH), 142.09 (OCHCH), 110.25 (CHCO), 108.35 (CHCHO), 62.33 (d, J = 6.2 Hz, CH_2CH_3), 62.01 (d, J = 6.1 Hz, CH_2CH_3), 56.27 (d, J = 7.5 Hz, CH_2CCH), 31.23 (d, J = 5.4 Hz, CH_2CHP), 31.03 (d, J_{P-C} = 216.7 Hz), 16.43 (CH_3), 16.37 (CH_3). ^{31}P NMR δ 22.40. IR(neat, cm^{-1}) 3113, 2983, 2931, 2908, 1505, 1243, 1017, 796. HRMS-EI (m/z): calcd for $C_{11}H_{19}NO_4P$ (M + H$^+$): 260.1052; found: 260.1058.

*4.2.5. Diethyl (1-(1-hydroxybutan-2-yl)aziridin-2-yl)phosphonate (**4e** and **4e'**)*

4e: Colorless oil, $[\alpha]_D^{21}$ = +38 (c, 0.1, CH_2Cl_2), (EtOAc/MeOH = 10:1, R_f = 0.32), yield 33% (69 mg, 0.274 mmol); ^{1}H NMR δ 4.20–4.01 (m, 4H), 3.68–3.53 (m, 2H), 3.33 (s, 1H), 2.01 (dd, J = 8.9, 3.6 Hz, 1H), 1.72 (ddd, J = 20.5, 6.6, 3.6 Hz, 1H), 1.57 (t, J = 7.1 Hz, 1H), 1.50–1.42 (m, 2H), 1.37–1.32 (m, 1H), 1.30 (t, J = 7.1 Hz, 3H), 1.26 (t, J = 7.1 Hz, 3H), 0.88 (t, J = 7.5 Hz, 3H). ^{13}C NMR δ 72.52 (d, J = 6.8 Hz, $CHCH_2OH$), 65.52 (CH_2OH), 63.01 (d, J = 6.0 Hz, CH_2CH_3), 62.08 (d, J = 6.6 Hz, CH_2CH_3), 31.11 (d, J_{P-C} = 218.3 Hz), 29.53 (d, J = 5.9 Hz, CH_2N), 24.65 (CH_2CHN), 16.35 (d, J = 2.8 Hz, CH_3CH_2O), 16.30 (d, J = 2.2 Hz, CH_3CH_2O), 10.47 (CH_3CH_2CH). ^{31}P NMR δ 23.84. IR(neat, cm^{-1}) 3403, 2977, 2933, 2877, 1233, 1019, 965, 795. HRMS-EI (m/z): calcd for $C_{10}H_{23}NO_4P$ (M + H$^+$): 252.1364; found: 252.1359. **4e'**: Colorless oil, $[\alpha]_D^{21}$ = +44 (c, 0.1, CH_2Cl_2), (EtOAc/MeOH = 10:1 R_f = 0.25), yield 45% (94 mg, 0.374 mmol); ^{1}H NMR δ 4.15–4.00 (m, 4H), 3.68–3.55 (m, 2H), 2.37 (s, 1H), 2.09 (dd, J = 9.1, 3.6 Hz, 1H), 1.67 (t, J = 7.1 Hz, 1H), 1.64–1.57 (m, 1H), 1.55–1.43 (m, 2H), 1.37–1.31 (m, 1H), 1.31–1.28 (m, 3H), 1.28–1.24 (m, 3H), 0.89 (t, J = 7.5 Hz, 3H). ^{13}C NMR δ 73.08 (d, J = 6.7 Hz, $CHCH_2OH$), 63.85 (CH_2OH), 62.52 (d, J = 6.4 Hz, CH_2O), 62.00 (d, J = 6.3 Hz, CH_2O), 31.34 (CH_2N), 29.73 (d, J_{P-C} = 219.5 Hz), 23.93 (CH_2CHN), 16.45 (d, J_{P-C} = 5.9 Hz, CH3CH2), 16.39 (d, J_{P-C} = 6.1 Hz, CH_3CH_2), 10.26 (CH_3CH_2CH). ^{31}P NMR δ 22.86. IR(neat, cm^{-1}) 3394, 2979, 2931, 2878, 1233, 1019, 964, 795. HRMS-EI (m/z): calcd for $C_{10}H_{23}NO_4P$ (M + H$^+$): 252.1364; found: 252.1354.

*4.2.6. Diethyl 1-(1-phenylethyl) aziridin-2-ylphosphonate (**4f** and **4f'**)*

4f: Colorless oil, $[\alpha]_D^{21}$ = +44 (c, 0.1, CH_2Cl_2), (EtOAc, R_f = 0.38), yield 49.2% (225 mg, 0.794 mmol); ^{1}H NMR δ 7.34–7.08 (m, 5H), 4.21–4.01 (m, 4H), 2.35 (q, J = 6.5 Hz, 1H), 1.97 (dd, J = 8.9, 3.3 Hz, 1H), 1.55 (ddd, J = 19.3, 6.8, 3.6 Hz, 1H), 1.45 (t, J = 7.0 Hz, 1H), 1.39 (d, J = 6.5 Hz, 3H), 1.29 (t, J = 7.0 Hz, 6H). ^{13}C NMR δ 143.79 (Ph), 128.28 (Ph), 127.14 (Ph), 126.58 (Ph), 70.97 (d, J = 7.1 Hz, CHPh), 62.41 (d, J = 6.5 Hz, CH_2OP), 62.19 (d, J = 6.3 Hz, CH_2OP), 32.59 (d, J_{P-C} = 218.6 Hz, CHP), 31.53 (d, J = 5.2 Hz, CH_2N), 23.54 (CH_3CH), 16.52 (d, J_{P-C} = 5.7 Hz, CH_3CH_2), 16.46 (d, J_{P-C} = 5.8 Hz, CH_3CH_2). ^{31}P NMR δ 22.76. IR(neat, cm^{-1}) 3060, 2978, 2929, 2906, 1245, 1021, 961. HRMS-EI (m/z): calcd for $C_{14}H_{21}NO_3PNa$ (M + Na$^+$): 306.1235; found: 306.1237. **4f'**: Colorless oil, $[\alpha]_D^{21}$ = +26 (c, 0.1, CH_2Cl_2), (EtOAc, R_f = 0.14), yield 32.6% (149 mg, 0.526 mmol); ^{1}H NMR δ 7.40–7.10 (m, 5H), 3.96–3.54 (m, 4H), 2.32 (q, J = 6.5 Hz, 1H), 2.19 (dd, J = 9.1, 3.6 Hz, 1H), 1.60 (t, J = 7.0 Hz, 1H), 1.52–1.42 (m, 1H), 1.40 (d, J = 6.6 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H), 1.03 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 143.21 (Ph), 128.23 (Ph), 127.34 (Ph), 127.13 (Ph), 71.42 (d, J = 6.9 Hz, CH_3CH), 62.14 (d, J = 6.2 Hz, CH_2CH_3), 61.48 (d, J = 6.0 Hz, CH_2CH_3), 31.97 (d, J = 5.2 Hz, CH_2N), 31.28 (d, J_{P-C} = 216.4 Hz, CHP), 22.93 (CH_3CH), 16.30 (CH_3CH_2), 16.23 (CH_3CH_2). ^{31}P NMR δ 21.49. IR (neat, cm^{-1}) 3060, 2978, 2929, 2906, 1246, 1022, 954. HRMS-EI (m/z): calcd for $C_{14}H_{21}NO_3PNa$ (M + Na$^+$): 306.1235; found: 306.1225.

*4.3. Aziridin-2-ylphosphonate (**4g**)*

Diethyl 1-benzylaziridin-2-ylphosphonate (**4a**, 50 mg, 0.19 mmol) and Pd–C (25 mg 10%) was stirred in CH_3OH (0.63 mL) under N_2 atmosphere. Then H_2 gas filled into the reaction flask having a baloon attached to it. After stirring for 30 min tlc analysis showed no starting material. The reaction mixture was filtered through celite and the solvent was removed by rotary evaporator to give pure compound **4g** (28.6 mg, 0.160 mmol) in 85% yield as a colorless oil. ^{1}H NMR δ 4.11–3.91 (m, 4H), 1.89 (d, J = 11.1 Hz, 1H), 1.79–1.61 (m, 2H), 1.30–1.15 (m, 6H). ^{13}C NMR δ 62.03 (t, J = 5.5 Hz), 22.54 (d, J = 2.4 Hz), 21.97 (d, J_{P-C} = 195.6 Hz), 16.38 (CH_3), 16.32 (CH_3). ^{31}P NMR δ 27.15. IR (neat, cm^{-1}) 3450, 3256, 2983, 2909, 1235, 1018, 958. HRMS-EI (m/z): calcd for $C_6H_{15}NO_3P$ (M + H$^+$): 180.0783; found: 180.0789.

*4.4. Synthesis of 1,2-dibromoethylphosphonate (**2**)*

Diethyl vinylphosphonate (**1**, 0.2 mL, 1.24 mmol) was added into a pre-dried two necked flask and was dissolved in CH_2Cl_2 (13 mL) and the reaction mixture cooled to 0 °C Br_2 (0.830 mL, 1.67 mmol, in 2.4 mL CH_2Cl_2) was added to this solution. After 30 min stirring at rt, tlc showed no starting material. The reaction mixture was applied directly to column chromatography (EtOAc) to yield **2** (382 mg, 1.18 mmol) in 95% yield as a light yellow oil. ^{1}H NMR δ 4.26–4.12 (m, 4H), 4.03–3.88 (m, 2H), 3.61–3.48 (m, 1H), 1.33 (t, J = 7.0 Hz, 6H). ^{13}C NMR δ 64.01 (d, J = 7.0 Hz, CH_2CH_3), 63.70 (d, J = 6.9 Hz, CH_2CH_3), 41.88 (d, J_{P-C} = 150.8 Hz), 31.72 (CH_2Br), 16.37 (CH_3CH_2), 16.32 (CH_3CH_2). ^{31}P NMR δ 15.27.

*4.5. Synthesis of aziridinyl phosphonates **4a–4g** by (Method B)*

Diethyl 1,2-dibromoethylphosphonate (**2**, 329 mg, 1.02 mmol) was weighed into a pre-dried two necked flask and dissolved by adding CH_3CN (1.85 mL). After adding Et_3N (0.170 mL, 1.22 mmol) at rt, white solids were observed. Then the amine (3.05 mmol) was added and the resulting mixture was refluxed at 80–85 °C for 3 h. At the end of this time, the reaction mixture was treated with 0.1 N HCl (10 mL) and CH_2Cl_2 (10 mL) was added. Two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography on silica gel using hexane:ethyl acetat (1:1 mixture) as the eluent.

4.6. Antibacterial assays

The prepared compounds **4a**–**g** were evaluated for their antibacterial activities against *Bacillus subtilis*, *Escherichia coli* DH5α, isolate Fs48 (*Gordonia* spp), Fs30 (*Brevundimonas* spp), Fs24 (*Kocuria* spp) by performing disc diffusion assays [20]. The 100 μl volumes from liquid cultures were spreaded onto nutrient agar in plates (Merck, Germany). A 100 μl volume from each test compound (we assumed that 1 μl compound is 1 μg) was dissolved in 400 μl 25% DMSO (DMSO final concentration was 20%). A 50 μl volumes from DMSO dissolved test compounds were incorporated in sterile disc filters (Whatman No. 1). The discs containing test compound and only 25% DMSO (control) were introduced into the middle of the bacteria inoculated agar surfaces in petri plates. The cultures were incubated 24 h at 28 °C. The experiments were performed in triplicate. Ampicillin and Streptomycin were used as the reference drugs (10 μg per disk). The results were recorded for each tested compound as the average diameter of bacterial growth inhibition zones around the disks in mm (Table 2).

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