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EVALUATION OF ANTIBACTERIAL ACTIVITIES OF NOVEL AZIRIDINYL PHOSPHONATES

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Abstract: A new series of aziridines was synthesized in our laboratory, which displays potent antibiotic activities[1-3]. However, a practical synthesis by using the coupling method of this aziridines with either phosphonate or N-phtaloyl acide moiety can be converted into various derivatives. This work describes new results of our ongoing research targeting new derivatives of biological interest. All the compounds were screened for their antibacterial activity, they all showed comparable moderate to good growth inhibitory activity with reference to Tetracyclin and Gentamicin.

Keywords: Aziridines; Phosphonates; Antibacterial activity; Amino acids.

I. Introduction

Antibiotic resistance is a major problem in hospitals as well as in community settings [4]. Considering the ever growing antibiotic resistance developed by many bacteria, there is an immense need for new compounds with new mode of actions, for treatment of bacterial infections [5], the need for new antibiotics continues to be a still standing challenge [6]. Aziridines represent an important class of compounds that exhibit antibacterial activities against a wide range of bacteria [7]. They are also important in chemistry, since they are widely used as versatile and reactive synthetic intermediates, and precursors to more complex molecules [8].

A few aziridine derivatives have been isolated from natural sources too [9] and some of them exhibited biological activities, with potential both antibiotic and anticancer therapy [10]. Although many synthetic pathways have been designed leading to various aziridines, but only a limited number of studies are found for the synthesis of aziridinylphosphonates [11]. One of these methods, reported by Kim et al. is related to nitrene addition to cinnamoyl phosphonate derivatives to generate *N*-tosyl-2-aziridinylphosphonates in 81-89% yield [12]. Another synthesis reported by Stevens et al. gives rather specific *N*-vinylaziridinylphosphonate derivatives in 29-57% yield [13]. Besides, Davis et al. reported

the synthesis of chiral aziridinylphosphonates in 76% yield by reacting chiral sulfinimines with chloromethylphosphonates [14].

We have, already reported the synthesis of several aziridines, which some of them showed potent antibacterial activity against Gram-positive bacteria (Fig.1). In continuation our research program, to find new antibacterial agents for the treatment of infectious diseases, we set up this work with a view to developing a general method leading to aziridinylphosphonates and to investigate their antibacterial activity. Herein we would like to report the synthesis and in vitro antibacterial profile of a series of novel phosphonoaziridines as potent antibacterial agents against Gram-positive and Gram-negative bacteria. Target compounds were synthesized according to a protocol previously developed in our laboratory, that allowed access to compounds endowed (Fig.2) with interesting anti tumour activities [15-16]. By contrast with the above mentioned studies, we replaced the amino acids phtaloyl protecting group with a phosphonate moiety and, surprisingly, the biological activity of the novel aziridines shifted from antiviral to an antibacterial one.

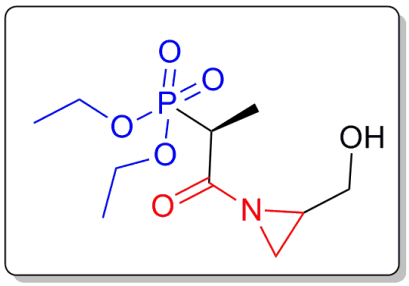


Fig.1: Aziridinylphosphonate

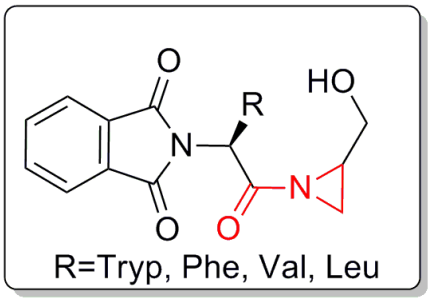


Fig.2: Peptidomimetic aziridine.

II. Experimental Section

All the reactions with dry solvents were carried out under dry nitrogen. THF was dried over sodium /benzophenone and freshly distilled before use; CH_2Cl_2 was distilled and dried over phosphorus pentoxide (P_2O_5). Triethylphosphite P((OEt)$_3$) was distilled before use under reduced pressure. I.R spectra were collected from a Mattson Genesis II FTIR. NMR spectra were recorded in $CDCl_3$ on a Bruker 300MHz instrument, using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in □ (ppm) and coupling constant (*J*) values in Hertz (Hz). ESI-MS data were recorded in the positive ion mode on a quadrupole instrument (Waters-Micromass ZQ). CG analysis was performed on a Shimadzu 17A CPG chromatograph using a 30m DB-35 column. Melting points were determined on an Electrothermal T1A F3.15A instrument. Column chromatography was performed on silica gel 230-270 mesh (Merck) using CH_2Cl_2, MeOH and ether. Elemental analysis was performed only for solids on a LECO CHN 900 instrument.

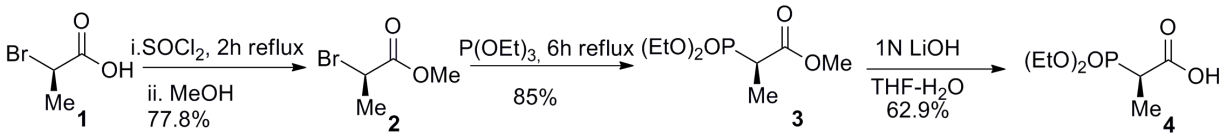
II. Antibacterial assays
II.1 Procedure

The prepared compounds **6a-d**, **8** and **9a-d** with Concentration, 32µg/ml were evaluated for their antibacterial activities against *Bacillus cereus* and *S.aureus* (Gram-positive), *Escherichia coli* and *Klebseilla pneumonie* (Gram-negative) by performing disc diffusion assays. The volumes from liquid cultures were spreaded onto nutrient agar in plates. The discs containing test compound and DMSO (control) were introduced into the middle of the bacteria inoculated agar surfaces in petri plates. The cultures were incubated 24h at 37°C. Tetracyclin and Gentamicin were used as the reference drugs. The results were recorded for each tested compound as the average diameter of bacterial growth inhibition zones around the disks in mm.

III. Results and Discussion

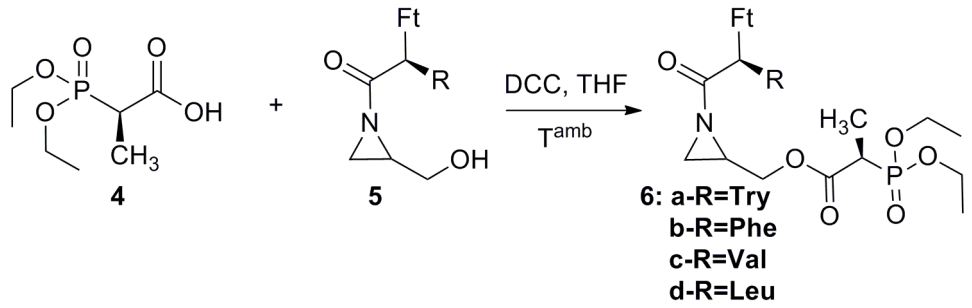
III.1. Chemistry of aziridine dervivatives

Due to the extensive use of aziridine in organic [17-19] and medicinal chemistry, several methodologies for the preparation of useful functionalized aziridines have been developed [20-25].
In our research work aimed at investigating the antibacterial activity of new aziridinyl-phosphonates, we embarked in the preparation of *N*-acyl-2-hydroxymethylaziridines **5** with interesting anti tumour activities to a protocol previously developed in our laboratory. Then coupling this aziridines **5** with a phosphonate moiety, leading to original functionalized aziridinylphosphonates.
Our synthetic pathways to phosphonate moiety and target aziridines **6a-d,** are presented in Schemes 1 and 2. The synthesis of **4** started with purified 2-bromopropionic methyl ester **2** that was reacted with triethylphosphite ($P(OEt)_3$), according to Arbuzov-Michaelis reaction to afford phosphonate **3** in good yield. **3** was hydrolyzed with 1N LiOH in tetrahydrofuran (THF)-water solution to yield 2-diethoxyphosphorylpropionic acid **4**.



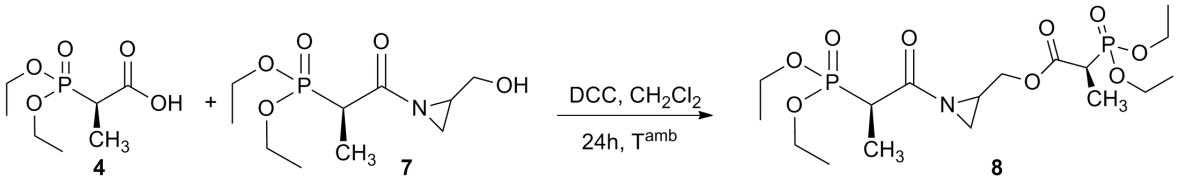
Scheme 1 : *Synthesis of diethylphosphonopropionic acid.*

Aziridines **5** were prepared according to a method previously reported by us. The latter were converted into target 2-phosphonomethylaziridines **6** after reaction at room temperature with **4**, in the presence of DCC as coupling agent to afford the novel 2-phosphonylaziridines in moderate to good yields 50-70%.



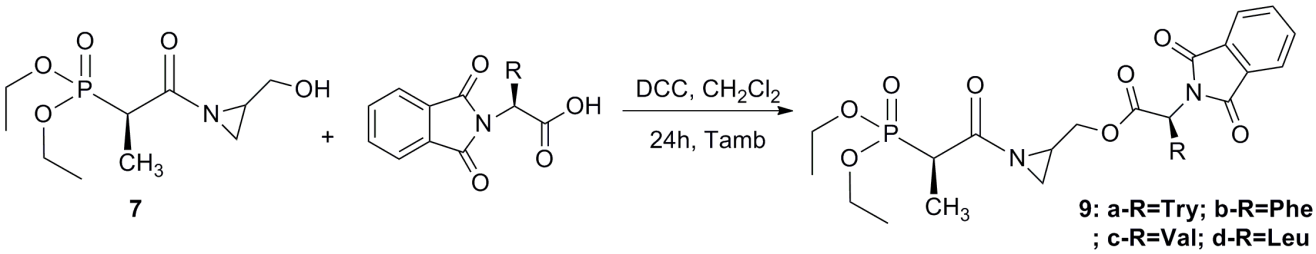
Scheme 2*: N-Phtalimido-N-acyl-2-phosphonomethylaziridines.*

Many approaches are developed in literature to obtain aziridinylphosphonate [26-28], but only few are describes for the synthesis of bisphosphonateaziridine. So the main idea was if the presence of two phosphonates moiety in the structure will improve or not the antibacterial activity of the novel bisphosphonateaziridine [29-31]. Aziridine **8**, was obtained with the same protocol describes above by coupling the phosphonate **4** with phosphonoaziridine **7** in the presence of DCC , to have aziridine **8** in moderate yield 50%.



Scheme 3 : *Synthesis of bisphosphonoaziridines.*

As phthalimidoamino acids were synthesised from a literature protocol[13], we engaged them in a coupling process with **7** to have a scaffold of *N*-phosphono-2-peptidylaziridines **9** that were obtained in 56-69% .



Scheme 4: *Synthesis of phosphono-methyl-N-acyl-2-N-phtalimidoaziridines.*

III.2. Antibacterial studies

Compounds **6 a-d**, **8** and **9 a-d** with Concentration, 32µg/ml, were screened for their antibacterial activity against multidrug resistant bacteria chosen from the Centre Hospitalo-Universitaire de Tlemcen/Algeria where they are responsible for a number of nosocomial infections. The bacteria of concern were namely *Bacillus cereus* and *Staphylococus aureus* (Gram-positive), *Escherichia coli* and *Klebseilla pneumonia (*Gram-negative). The screening was performed on disc diffusion assays. Tetracycline and Gentamicine (CT0056B, OXOID), were used as reference drugs. The results were recorded for each tested compound **6 a-d**, **8** and **9 a-d** (the experiment was repeated three times) as the average Diameter, mm of the inhibition zone of bacterial growth (Fig. 3).

Table 1 : The majority of the evaluated aziridine derivatives exhibited moderate to good activity as compared to reference antimicrobial drugs.

Aziridines		**Inhibition zone diameter (mm)**			
		B.cereus	*S.aureus*	*E.Coli*	*K.pneumoniae*
6	**a**	-	10 ±4	18±2	19±5
	b	-	11±2	17±3	21±3
	c	-	13±1	16±3	20±2
	d	-	12±2	15±2	17±4
7		12 ±5	19 ±3	22±4	29±3
8		10±4	15±3	25±5	35±2
9	**a**	11±2	11±4	12±4	25±2
	b	15±3	12±4	15±3	23±3
	c	15±3	10±4	10±3	20±5
	d	15±4	14±4	16±2	20±5
Tetracycline		20	20	17	19
Gentamicine		18	19	18	19

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 Fig.3 aziridinyl phosphonate **8** showed better activity than the other aziridines, against *Klebsiella pneumonia* and E. coli. Aziridine **7** with a free hydroxyl group presented good activity against both *Klebsiella pneumonia*

and *E.Coli.* From results displayed in Fig.3, we can assume that on the whole, our compounds are more active against Gram negative than Gram positive bacteria. Nevertheless, compound **6a-d** was inactive against *Bacillus cereus* while compounds **7**, **8** and **9a-d** showed a moderate inhibitory activity against the same bacterium.

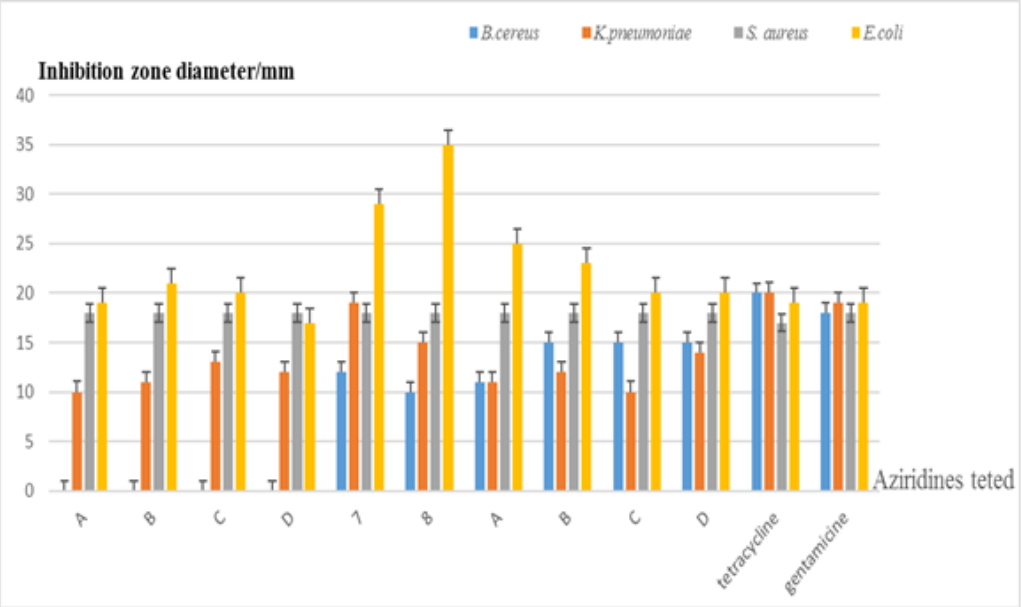


Fig .3. Antibacterial activities of aziridinylphosphonates.

From these results, it can be concluded that the substituent on the hydroxyl as well as the presence of two phosphonate moiety in aziridine ring, affects the antibacterial activity of these compounds, the most encouraging results being found against *Klebsiella pneumonia*.

IV. Conclusion

This work shed light on the fact that the biological activity of hydroxymethylaziridines, can be modulated by introducing various substituents on their basic structures. Moreover and besides showing interesting antibacterial activities, the series of novel compounds can be further improved to serve as potential drug against nosocomial diseases. The main advantages of the method developed in this study are the ease of availability of the starting materials, and the fact that the aziridination reaction proceeds at room temperature. As stated in the introduction, phtaloyl derivatives of our previous studies were active against breast cancer cells whereas phosphonate derivatives showed antibacterial activity. Therefore, work is going on for the diversification of the initial work, especially for the search of targeted cancer chemotherapy through the synthesis of hybrids.

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