Synthesis of a Novel Class of Phosphonoaziridines as Interesting Antibacterial Agents

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Abstract: Aziridines constitute an interesting class of organic compounds because of their reactivity and the facility they can be converted into various derivatives. Our group has developed a new class of aziridines that show interesting biological activities such as antitumor and anti bacterial. This work describes new results of our ongoing research targeting new derivatives of biological interest.

Keywords: Aziridines, aza-Wittig, cancer, phosphonates, protease inhibitors, strained heterocycles.

INTRODUCTION

Heterocycles structures are found in so many fields of scientific investigation - including organic, inorganic, bioorganic, agricultural, industrial, pharmaceutical, and medicinal chemistry, as well as material science- that their importance can hardly be overemphasized [1]. Therefore a long lasting effort is maintained towards the development of new synthetic protocols for the preparation of those compounds and their numerous derivatives.

Of particular importance are aziridines because of their reactivity. They are valuable synthetic intermediates that are widely used to access numerous drugs and biologically active products [2-4]. Many aziridine alkaloids show anticancer, antibacterial, and/or antimicrobial and antileishmanial activities [5-8]. Some among them behave as potential protease inhibitors [9-10]. Therefore, an assumption might be made that the presence of an aziridine moiety in natural as well as synthetic compounds structures is essential to the observed activities [11]. As a result, several syntheses are found in the literature and it is beyond the scope of this work to mention all of them [12-25].

The biological activity of aziridines is highly related to the establishment of covalent bond with DNA [26]. In a previous work we reported the synthesis of aziridinyl derivatives [27] that had antitumor activities against breast cancer cells [28]. Such a behaviour was likely due to their capacity to strengthen and modulate the immune system [29]. Thus, going on with our efforts to develop new biologically active derivatives we replaced the phthaloyl group with a phosphonyl one entails a shift of activities from antiviral to antibacterial ones. Unfortunately, the assays could not be extended to other available compounds of the same family, since the investigator in charge of biological tests left the laboratory. Therefore, the work presented here represents the synthesis of some aziridines that will serve as starting materials for the preparation of hybrids, of which work is on course, along with partial results about biological assays.

RESULT AND DISCUSSION

Syntheses

During our investigation, the target compound was 7, "N-(diethylphosphonopropionyl) -2-hydroxymethylaziridine (Fig. 1). Preliminary antibacterial assays revealed it to be endowed with interesting antibacterial activity against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Enterococcus faecalis. Needless to say that those strains are globally known to withstand treatment with common antibiotics and are the source of many nosocomial diseases.

It is worth mentioning that a simple replacement of a phthaloyl moiety with phosphonyl one entails a shift of activities from antiviral to antibacterial ones. Unfortunately, the assays could not be extended to other available compounds of the same family, since the investigator in charge of biological tests left the laboratory. Therefore, the work presented here represents the synthesis of some aziridines that will serve as starting materials for the preparation of hybrids, of which work is on course, along with partial results about biological assays.

![Fig. (1). Structures of Diethyl 1-(2-(hydroxymethyl)aziridin-1-yl)-1-oxopropan-2-ylphosphonate.](image-url)

The synthesis of 7 started from 2-bromopropionic methyl ester 2 that was previously distilled under reduced pressure
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to afford a colorless oil, before reacting with triethyl phosphite according to Arbuzov reaction, to afford the phosphonate 3 in good yield. 3 was hydrolyzed with a 1N LiOH in tetrahydrofuran (THF)-water solution to yield 2-(diethoxy phosphoryl)propionic acid 4.

The latter was converted into an acylazide 5, after reaction with ethyl chloroformate and sodium azide. The azide was used without further purification in the next step to yield the iminophosphorane. The generation of the non-isolated iminophosphorane was monitored by the evolution of nitrogen from the reaction solution.

Afterwards, glycidol alcoholate, in situ generated by treatment of glycidol with sodium hydride in dry ether, was dropwise added to the iminophosphorane solution. The reaction was left to proceed under nitrogen to yield the target compound. The reaction could be monitored by the progressive formation of a white solid corresponding to triphenylphosphine oxide, as could be checked later with an authentic commercial sample (Aldrich).

The conversion of an acyl azide into an aziridine is believed to occur according to mechanism we already suggested in our previous work [27]. After quenching the reaction mixture with an aqueous solution of ammonium chloride and removal of triphenylphosphine oxide, the compound was purified on a silica gel column using petroleum ether (b.p. 40-60°C)-dichloromethane. Satisfactory IR, ¹H-NMR, and ¹³C-NMR characteristics were obtained.

N-acylazide could also be prepared through a direct reaction of sodium azide in dimethylformamide (DMF) and with an acylchloride in dry dichloromethane, or according to a literature protocol [39].

As related to the synthesis of aziridines, we also prepared N-acyl-2-tosylmethylaziridine from O-tosylglycidol. The latter was first converted into an azido alcohol and further transformed into an aziridine by means of triphenylphosphine according to literature [40]. This compound showed more potent antibacterial activity as compared to compound 7. Probably the tosylate moiety could contribute to this enhanced activity, and this needs to be verified during further studies.


1. Synthesis of aziridin-2ylmethylbenzensulfonate 11

The O-protection of glycidol 8 with p-toluensulfonfyl chloride (TsCl) in the presence of triethylamine (TEA), led to 9 in good yield (Scheme 3). The solution of 9 in ethanol and water was treated with ammonium chloride and sodium azide to give the azido alcohol 10 that was reacted in the next step with a solution of triphenylphosphine in anhydrous tetrahydrofuran (THF) to provide 11 in high yield. Compound 11 was purified on a silica gel column eluted with dichloromethane (CH₂Cl₂)-methanol (MeOH) (v/v: 1:1).

2. Diethylphosphonopropionyl-2-tosylmethylaziridine 12

Diethylphosphonopropionic acid was reacted either with thionyl chloride in the presence of TEA to yield an acyl chloride that was reacted with 11 to give 12 (50%), or coupled in the presence of dicyclohexylcarbodiimide (DCC) with unprotected aziridine 11 to give the same compound in high yield (90%).

This strategy enabled us use 2-methyltosylate aziridine 11 as building block to obtain different functionalized aziridines. The same approach was successfully applied to generate aziridines from N-phtaloylamino acids 13. Phenylalanine and tyrosine were chosen as models, since in our initial work in this field [27], aziridine from the former amino acid showed the best biological profile [28], whereas tyrosine is claimed to behave as a residue of utmost importance in many receptors [41]. Derivatives 13 were prepared according to a modified literature procedure [42] and were recrystallized. They were further converted into aziridines 14 according to both methods previously described in the last section. After work-up, the phtaloyl group (Ft) could be removed from the protected amino acid moiety of 14a by treatment with hydrazine hydrate according to literature [43], affording 15a with a free amino group for a potential peptide growth.

Preliminary Biological Tests

Compound 7 was submitted to biological assays on Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922 (gram negative bacteria), Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212 (gram positive bacteria). The assays were carried out by disc diffusion on agar medium, where compound 7 showed medium to medium activity on Pseudomonas aeruginosa, and a weak one on Staphylococcus aureus. Gentamycin and Ciprofloxacin were used as references (Rahmoun, N. Unpublished results 2011. Détermination du pouvoir antibactérien d’une aziridène nouvellement synthétisée au Laboratoire COSNA).

As compared to the previous activity of phtaloyl aziridines, the results is interesting in such a way that it enables us modify or fix various groups on our aziridines to extend the scope of their biological activity. That is why compound 7 is already engaged in such a work. However, much work is still to be carried out on compound 7 and derivatives as related to assess as accurately as possible the Minimal Inhibitory Concentrations (MIC), the antibacterial effect versus concentration, as well as the biological pathway targeted by this aziridine and derivatives.

CONCLUSION

Numerous synthetic methods are found in the literature and allow easy accesses to various aziridines. It is worth
mention that most of those methods are based on the conversion of an amino group into the corresponding aziridine. By contrast, our synthetic procedure targets the carboxylic group and its transformation into acyl aziridines, while the amino group is either protected or replaced by a phosphonate. As compared to our initial work [27], the present study has allowed us develop a second class of aziridines of interesting biological activities, represented by compound 7. The phthaloyl derivatives were active against breast cancer cells [28] whereas phosphonate showed antibacterial activity. Work is going on for the diversification of the initial work, especially for the search of targeted cancer chemotherapy, through the synthesis of hybrids.

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$_2$Cl$_2$ was distilled and dried over phosphorus pentoxide (P$_2$O$_5$). Triethylphosphite (POEt)$_3$) 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 $\delta$ (ppm) and coupling constant (J) values in Hertz (Hz). GC 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$_2$Cl$_2$, MeOH and ether. Elemental analysis was performed only for solids on a LECO CHN 900 instrument.

Methyl-2-bromopropionate 2

To 2-bromopropionic acid (20 g, 0.13 mol), thionyl chloride (23.32g, 0.196mol) was added at room temperature. The reaction mixture was heated under reflux for 2 h. The mixture was cooled and methanol (0.261mol) was very slowly added with stirring and cooling, and the reaction was left for 30min. Excess methanol and thionyl chloride were removed in vacuo, and dichloromethane (50 ml) was added. The organic layer was washed respectively with a 5% solution of sodium bicarbonate (20ml), a saturated solution of sodium chloride (20 ml) and then dried over calcium sulfate. The dry solution was filtered and evaporated under vacuum to afford a residue that was purified by distillation under reduced pressure to afford a colorless oil (77.77%). bp = 50°C, 12mm Hg); IR cm$^{-1}$ neat: 1743.95 (C=O), 1160.03 (C–O), 673.81(C–Br). GC analysis, retention time (Rt) = 7.22 mn. $^1$H RMN, $^\delta$ CDCl$_3$ 300MHz: 1.95(d, J= 6.5Hz, 3H, CH$_3$), 3.69 (s, 3H, OCH$_3$), 4.49 (s, 2H, BrCH$_2$CO). $^{13}$C RMN, $^\delta$ CDCl$_3$: 23.90, 50.87, 160.02, 190.90

Methyl 2-(diethoxy phosphoryl)propanoate 3

Methyl 2-bromopropionate (35mmol) was introduced under nitrogen into a dry flask and then heated on reflux at 120 °C in a fume cupboard. Triethylphosphite (35mmol) was slowly added over a period of 35 mn. When the addition was completed the reaction mixture was refluxed for 6h and checked for completion by gas chromatography. At the end, the mixture was distilled under vacuo to afford a colorless oil. Yield 85%; bp = 140°C, 12mmHg; GC: retention time 24.25mn; $R_f$=0.32 on silica gel plate F$_{254}$ (Merck) AcOEt/CH$_2$Cl$_2$ (5:1). IR cm$^{-1}$ neat 1740.16 (C=O), 1165.95 (C–O), 1024.98 (P=O), 965.51 (P–O).
2-(diethoxy phosphoryl)propionic acid 4

To a mixture of tetrahydrofuran and water (v/v: 81ml/40ml), ester (23mmol) was introduced under magnetic stirring and an aqueous solution of 1N LiOH (24mmol) was added. The mixture was stirred for 1h at 0°C and left to stand overnight at room temperature. It was extracted with ethyl acetate; the aqueous layer was collected, and acidified with a 1N HCl aqueous solution to pH 2. Extraction was then carried out with ethyl acetate; the aqueous solution was saturated with salt, and extracted for a last time. The organic extracts were combined, dried over CaSO₄, filtered and concentrated in vacuo. Yield 62.96%. IR cm⁻¹ neat 3405.66 (P=O). 1H RMN, CDCl₃: 3.19, 16.29, 46.48, 176.99.

Diethyl 1-azido-1-oxopropan-2-ylphosphonate 5

To a well stirred and cooled solution of carboxylic acid (13,5mmol), dry CH₂Cl₂, and dry TEA (1.47g, 14,6mmol) were added under nitrogen. A solution of ethyl (13,5mmol), dry CH₂Cl₂, and dry TEA (1.47g, 14,6mmol) was added. The mixture was stirred for 1h at 0°C and left to stand overnight. It was extracted with ethyl acetate; the aqueous solution was saturated with salt, and extracted for a last time. The organic extracts were combined, dried over CaSO₄, filtered and concentrated in vacuo. Yield 62.96%. IR cm⁻¹ (KBr pellet): 3473.52(O–H), 1669.65(C=O), 1022.52(P=O).

Oxiran-2-ylmethy-1-methylbenzenesulfonate 9

Dry triethylamine (10.9g, 0.1081mol) and p-toluenesulfonyl chloride (20.61g, 0.11mol) were added to a stirred solution of glycidol (2g, 27,02mmol) in dry CH₂Cl₂ under nitrogen at 0°C. The reaction mixture was stirred at room temperature overnight. After the reaction completion, the resulting mixture was filtered through silica gel and washed with ethyl acetate. The organic layer was dried over sodium sulfate, and the solvent removed under reduced pressure. The resulting compound was stored in the cold under dry nitrogen. The resulting compound was stored in the cold under dry nitrogen. The crystals were filtered off under suction.

Oxiran-2-ylmethyl 4-methylbenzenesulfonate 9

Microanalysis: calcd for C₁₀H₂₀NO₅P: C 45.28%, H 7.60% N 5.28%. Found: C 45.24%, H 7.59, N 5.25%
brine and dried over anhydrous sodium sulfate. Filtration and concentration in vacuo afforded the azido alcohol (57%). IR cm⁻¹: 3387.31 (O–H), 1366.79 (S=O), 1177.09 (C–O), 932.53 (S=O), 722.28–696.32 (OTs).

**Method 2** was obtained in good 90% yield. The residue was purified on silica gel column using CH₂Cl₂-MeOH (1:1). IR cm⁻¹: 3387.31 (O–H), 1356.78 (S=O), 1177.09 (C–O), 932.53 (S–O), 722.28–696.32 (OTs).

Aziridin-2-ylmethylbenzenesulfonate 11

To a solution (1.25g, 4.99mmol) of azido alcohol in anhydrous THF, a solution of triphenylphosphine (1.33g, 4.99mmol) in anhydrous THF was added dropwise. The mixture was refluxed for 2 h until all the solids dissolved and then cooled to room temperature and afterwards in an ice bath. The solid was filtered under suction and recrystallized from ethanol and water (4:1). The compound was identical to an authentic commercial sample from Aldrich. mp = 183°C Yield: 83%; IR (KBr): 3269.43 (O–H), 1771.55 (C=O phtaloyl), 1748.50 (C=O), 1102.05 (C–O), 972.25 (S–O), 723.93–683.38 (OTs).

**N-Phtalimidophenylalanine 13a**

Phenylalanine (0.121mol) was suspended in glacial acetic acid (100mL) and phthalic anhydride (0.12 mol) was added. The mixture was refluxed for 2 h until all the solids dissolved and then cooled to room temperature and afterwards in an ice bath. The solid was filtered under suction and recrystallized from ethanol and water (4:1). The compound was identical to an authentic commercial sample from Aldrich. mp = 183°C Yield: 83%; IR (KBr): 3269.43 (O–H), 1771.55 (C=O phtaloyl), 1748.50 (C=O), 1102.05 (C–O).

**N-Phthalimidotyrosine 13b**

The same procedure for the synthesis as above mentioned for 13a, except for the reflux time duration that was 24h.

**Diethylphosphonopropionyl-2-tosylmethylaziridine 12**

IR (KBr): 1728.87 (C=O), 1366.79 (S=O), 1119.96 (C=O), 1023.46 (P=O), 932.07 (S=O), 723.31–695.46 (OTs).

**1H RMN δ CDMC**: 300MHz: 1.27(d, J= 5.1Hz, 3H, CH₃(CH)), 1.29(t, J = 7.1Hz, 6H, CH₃(CH₂OP)), 1.70(d, J= 4.5Hz, 2H, CH₂N), 1.75–1.79(m, 1H, CHN), 2.34(s, 3H, CH₃Ts), 3.68(dd, J=13.2Hz, J=4.5Hz, CH₂N), 3.88(dd, J=13.2Hz, J=4.5Hz, 1H, CH₂N), 4.01(q, J = 3.9Hz, 1H, CH(CH₃)), 4.09(q, J = 2.4Hz, 4H, POCH₂CH₃), 7.75(d, J= 3Hz, 2H, Ar), 7.46(d, J= 3Hz, 2H, Ar).

**13C RMN δ CDMC**: 43.8, 16.29, 21.28, 29.35, 30.56, 42.51, 61.78, 73.58, 128.25, 130.48, 144.76, 177.19.

N-phthalimidophenylalanine 13a

Phenylalanine (0.121mol) was suspended in glacial acetic acid (100mL) and phthalic anhydride (0.12 mol) was added. The mixture was refluxed for 2 h until all the solids dissolved and then cooled to room temperature and afterwards in an ice bath. The solid was filtered under suction and recrystallized from ethanol and water (4:1). The compound was identical to an authentic commercial sample from Aldrich. mp = 183°C Yield: 83%; IR (KBr): 3269.43 (O–H), 1771.55 (C=O phtaloyl), 1748.50 (C=O), 1102.05 (C–O).

**1H RMN δ CDMC**: 300MHz: 3.6(d, CH₂, J=5.5Hz), 5.24(t, CH₂, J=7.5Hz), 7.25(s, 5H, Ph), 7.79(s, 4H, Fıt), 7.91(s, CO₂H).

N-Phthalimidotyrosine 13b

The same procedure for the synthesis as above mentioned for 13a, except for the reflux time duration that was 24h.

**mp=149°C**. 1H RMN δ CDMC , 300MHz: 3.21(dd, J= 8.5Hz, 4.1Hz, 1H, NCH-CH₂), 3.46(dd, J= 8.5Hz, 4.1Hz, 1H, NCH-CH₂), 4.80(m, 1H, NCH), 5.30(s, 1H, OH), 6.65(d, J= 6.5Hz, 2H, ArOH), 7.08(d, J= 6.5Hz, 2H, ArOH), 7.80–7.84 (m, 4H, Ar).

**13C RMN δ CDMC**: 33.48, 60.86, 112.75, 126.19, 120.64, 127.18, 128.17, 152.65, 171.69.

(1-[(2-(1,3-dioxoisoindolin-2-yl)-3-phenylprop-2-enoyl)aziridin-2-yl)methyl 4- methylbenzenesulfonate 14a

Pasty product. 1771.75(C=O Fıt), 1710.04 (C–O azidine), 1349.17(S=O), 1186.10 (C–O), 723.93–683.38 (OTs).

**1H RMN δ CDMC**: 300MHz: 1.66(dd, J= 8.7Hz, J= 4.5Hz, 1H, NCH-CH₂), 1.41(dd, J= 8.7Hz, J= 4.5Hz, 1H, NCH), 1.71(m, 1H, NCH), 2.31(s, 3H, CH₃Ph), 3.21–3.84(m, 4H, CH₂Ph, CH₂O), 5.16(m, 1H, CONH), 7.23–7.72(m, 3H, Ar), 4.36(d, J=6Hz, 2H), 7.81–7.86(m, 4H, Ar).

**13C RMN δ CDMC**: 18.29, 26.38, 27.57, 32.01, 57.28, 70.56, 120.65, 122.85, 126.52, 129.17, 136.18, 141.38, 164.86. Elemental analysis: caled for C₂₇H₂₃N₄O₆S: C 64.27%, H 4.79%, N 5.55%. Found: C 63.87%, H 4.75%, N 6.01%.

(1-[(2-(1,3-dioxoisoindolin-2-yl)-3-(4-hydroxyphenyl)prop-2-enoyl)aziridin-2-yl]methyl 4- methylbenzenesulfonate 14b

mp = 201°C. IR (KBr): 3282.41 (O–H), 1775.61(C=O of Fıt), 1626.75 (C=O of azidine), 1311.81(S=O), 1127.16 (C–O), 972.25 (S=O), 723.93–683.38 (OTs).
**REFERENCES**


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