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Synthesis of 1-(2-aminoethylsulfonyl)-2-phosphorylpyrrolidines *via* consecutive Arbuzov and aza-Michael reactions and their antitumor activity

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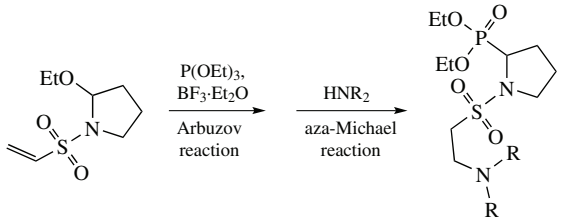
1-(2-Aminoethylsulfonyl)-2-phosphorylpyrrolidines have been synthesized *via* boron trifluoride-catalyzed Arbuzov reaction of 2-ethoxy-1-(vinylsulfonyl)pyrrolidine with triethyl phosphite followed by aza-Michael reaction of thus obtained 2-phosphoryl-1-(vinylsulfonyl)pyrrolidine with secondary amines. The cytotoxicity of the prepared 1-(2-aminoethylsulfonyl)-2-phosphorylpyrrolidines against M-Hela tumor cell line is comparable with that of tamoxifen, whereas the cytotoxicity against normal cell line is twofold lower.



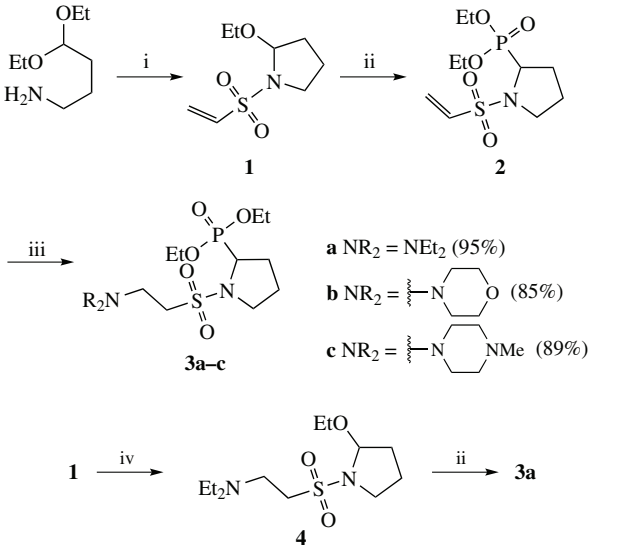
Pyrrolidine core is a structural motif of many biologically active compounds[1,2] and one of the most frequently occurring heterocyclic scaffolds in approved drugs.[3] At the same time, α-amino phosphonic acids as analogues of the natural amino acids play an important role in the investigation of biologically active molecules.[4–6] Among them, pyrrolidine derivatives possessing phosphoryl moiety are of a special interest due to their unique biological properties, including bactericidal, fungicidal and herbicidal activities.[7–9] Some of them were patented as endothelial differentiation gene (EDG) receptor agonists.[10] Oligopeptides with phosphorylproline fragment are of interest as inhibitors of various proteases[11–14] and HIV-1 proteinase.[15,16] A nucleoside containing phosphorylpyrrolidine fragment can be used in the treatment of hepatitis B.[17]

One of the common pathways to 2-phosphoryl-substituted pyrrolidines is based on Lewis acid-catalyzed Arbuzov-type reaction of 2-alkoxypyrrolidines with various trialkyl-[18–20] and triaryl phosphites.[19] Two main disadvantages of this approach are the cumbersome and multi-stage synthesis of the starting 2-alkoxypyrrolidine derivatives and a lack of possibility of further modification of those 2-phosphorylpyrrolidines.

Recently, we reported an easy one-pot and one-step access to 2-ethoxy-1-(vinylsulfonyl)pyrrolidine and converted it into various 2-arylpyrrolidines.[21–23] The presence of activated double C=C bond allows one to further modify the latter *via* aza-Michael reaction giving rise to biologically active water-soluble compounds. Herein, we report on the successful extension of this protocol to the synthesis of amine-containing 2-phosphoryl-1-sulfonylpyrrolidines, as well as on the study of their cytotoxicity against normal and tumor cells.

First, starting 2-ethoxy-1-(vinylsulfonyl)pyrrolidine **1** was obtained *via* the reaction of 2-chloroethane-1-sulfonyl chloride with 4,4-diethoxybutane-1-amine as described previously[21] (Scheme 1). Two further key stages included reaction of 2-ethoxypyrrolidine **1** with triethyl phosphite in the presence of boron trifluoride catalyst and aza-Michael addition of various amines at sulfonyl-activated double bond. The order of these stages could be changed, thus providing two routes to the target nitrogen-containing 2-phosphoryl-1-sulfonylpyrrolidines **3**.

The reaction of 2-ethoxypyrrolidine **1** with diethylamine resulted in 2-(diethylamino)ethane-1-sulfonamide derivative **4** (see Scheme 1) in excellent (92%) yield. However, subsequent reaction of compound **4** with triethyl phosphite led to the expected 2-phosphorylpyrrolidine **3a** in rather low yield (14%). The alternative route was tried out afterwards. Reaction of compound **1**



Scheme 1 *Reagents and conditions*: i, $Cl(CH_2)_2SO_2Cl$, CH_2Cl_2, room temperature, then Et_3N; ii, $P(OEt)_3$, $BF_3·Et_2O$, CH_2Cl_2, room temperature, 240 h; iii, HNR_2, CH_2Cl_2, room temperature, 48 h; iv, $HNEt_2$, CH_2Cl_2, room temperature, 24 h.

Table 1 The cytotoxicity and hemolytic activity of compound **3a**.

Compound	IC_{50}/μM			Hemolysis (%)		
	Tumour cell lines		Normal cell line	Concentration/μM		
	M-Hela	MCF_7	Chang liver	100.0 ±7.8	50.0 ±4.3	10.0 ±0.8
3a	15.1±1.3	53.0±4.1	82±6.9	0.5	0.1	0
Tamoxifen	28.0±2.5	25.0±2.2	46.2±3.5			

with 2 equivalents of triethyl phosphite in the presence of boron trifluoride afforded [1-(vinylsulfonyl)pyrrolidin-2-yl]phosphonate **2** in 34% yield. Next, aza-Michael reaction of compound **2** with diethylamine gave diethyl (pyrrolidin-2-yl)phosphonate **3a** in 95% yield.† Thus, the overall yield of compound **3a** appeared to be higher in this case (32% *vs*. 13%). Similarly, compounds **3b**,**c** possessing saturated heterocyclic moieties at the sulfonylamide fragment were obtained in 28–30% overall yields.

Cytotoxicity of 2-phosphorylpyrrolidine **3a** against normal and tumor cells was estimated by counting viable cells using the CytellCellImaging multifunctional system (GE HelthcareLifeScience, Sweden) and the CellViabilityBioApp application, which allowed us to accurately calculate the number of cells and evaluate their viability based on fluorescence intensity.[24] M-Hela clone 11 and MCF7 tumor cell lines and normal liver cells (Changliver) were used for experiments. The cytotoxicity was studied at concentrations recommended for screening for new antitumor agents (100–1 μM). Compound **3a** selectively acts on the M-Hela cell line (Table 1). The IC_{50} values of pyrrolidine **3a** are comparable with those of tamoxifen reference drug. It should especially be noted that pyrrolidine derivative **3a** was significantly less toxic than tamoxifen against normal cell line. Additionally, the hemolytic activity of compound **3a**, tested in 100–10 μM range using method described previously,[25] has been found to be negligible (see Table 1).

† *Diethyl [1-(vinylsulfonyl)pyrrolidin-2-yl]phosphonate* **2**. To a mixture of 1-sulfonylpyrrolidine **1** (1.00 g, 5.00 mmol) and triethyl phosphite (1.62 g, 10 mmol) in dry benzene (30 ml), boron trifluoride etherate (1.38 g, 10 mmol) was added in inert atmosphere (Ar). The mixture was kept at room temperature for 10 days. Brine (50 ml) was then added, and the mixture and stirred vigorously for 15 min. The water layer was extracted with dichloromethane (3×50 ml). The organic extracts were combined, dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The residue was chromatographed on 230–400 mesh silica with hexane/ethyl acetate (1 : 2) to give product **2** as yellowish oil. Yield 0.5 g (34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.26 (td, 6H, *J* 7.1, 4.3 Hz), 1.74–1.90 (m, 1H), 2.07–2.22 (m, 3H), 3.24–3.34 (m, 1H), 3.36–3.44 (m, 1H), 3.95–4.02 (m, 1H), 4.04–4.21 (m, 4H), 5.93 (d, 1H, *J* 9.9 Hz), 6.20 (d, 1H, *J* 16.6 Hz), 6.50 (dd, 1H, *J* 16.5, 9.9 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 16.3 (d, *J* 5.9 Hz), 16.4 (d, *J* 5.7 Hz), 24.9 (d, *J* 1.3 Hz), 27.3, 48.8 (d, *J* 1.3 Hz), 55.2 (d, *J* 169.5 Hz), 62.4 (d, *J* 6.9 Hz), 63.2 (d, *J* 7.2 Hz), 127.8, 133.2. ^{31}P NMR (160 MHz, $CDCl_3$) δ: 23.3. ESI-MS, *m/z*: 320 $[M+Na]^+$ (calc. for $C_{10}H_{20}NO_5PS$, *m/z*: 320).

Diethyl [1-(2-diethylaminoethylsulfonyl)pyrrolidin-2-yl]phosphonate **3a**. To a solution of [1-(vinylsulfonyl)pyrrolidin-2-yl]phosphonate **2** (0.15 g, 0.5 mmol) in dry dichloromethane (5 ml), diethylamine (0.5 mmol) was added dropwise. The mixture was stirred at room temperature for 2 days. The solvent was evaporated *in vacuo*, the residue was washed with diethyl ether and dried *in vacuo* (room temperature, 0.1 Torr, 2 h) to give the target compound **3a**. Yield 0.18 g (95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91–1.15 (m, 6H), 1.29–1.43 (m, 6H), 1.86–1.96 (m, 1H), 2.08–2.27 (m, 3H), 2.47–2.64 (m, 4H), 2.97–3.06 (m, 2H), 3.24–3.38 (m, 3H), 3.65–3.77 (m, 1H), 4.13–4.22 (m, 4H), 4.25–4.35 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 11.9, 16.4, 25.5, 27.5, 46.2, 46.9, 49.2, 49.9, 54.6 (d, *J* 168.8 Hz), 62.6. ^{31}P NMR (160 MHz, $CDCl_3$) δ: 24.4. ESI-MS, *m/z*: 371 $[M+H]^+$ (calc. for $C_{14}H_{31}N_2O_5PS$, *m/z*: 371).

In conclusion, we have developed two-stage approach to 1-sulfonyl-2-phosphorylpyrrolidine derivatives bearing amine fragment based on consecutive Arbuzov and aza-Michael reactions of 2-ethoxy-1-(vinylsulfonyl)pyrrolidine. The cytotoxicity of 1-sulfonylpyrrolidine **3a** has been tested to evaluate the feasibility of these compounds as novel antitumour agents. Notably, the cytotoxicity of tested compound against M-Hela tumour cell line is comparable to that of tamoxifen. At the same time, the cytotoxicity against normal cell line is two-fold lower, which makes this class of compounds interesting for further studies.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.11.028.

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