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EVALUATION OF (*E*)-2-CHLOROVINYLSULFONES AS NOVEL CLASS OF CYTOTOXIC AGENTS AND HIGHLY (*E*)-STEREOSELECTIVE ADDITION OF N-, S- AND Se-NUCLEOPHILES TO (*E*)-2-CHLOROVINYLSULFONES UNDER PHASE TRANSFER CATALYSIS CONDITIONS

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ABSTRACT:
Novel phase transfer catalytic (PTC) method for the conjugate addition-elimination of N-, S- and Se-nucleophiles to (*E*)-2-chlorovinylsulfones has been developed. Products were isolated in yields up to 98%. (*E*)-2-Chlorovinylsulfones exhibit very high cytotoxicity against MG-22A and HT-1080 cancer cell lines.

Keywords: 2-chlorovinylsulfones; conjugate addition; phase transfer catalytic system; toxicity; cytotoxicity.

INTRODUCTION

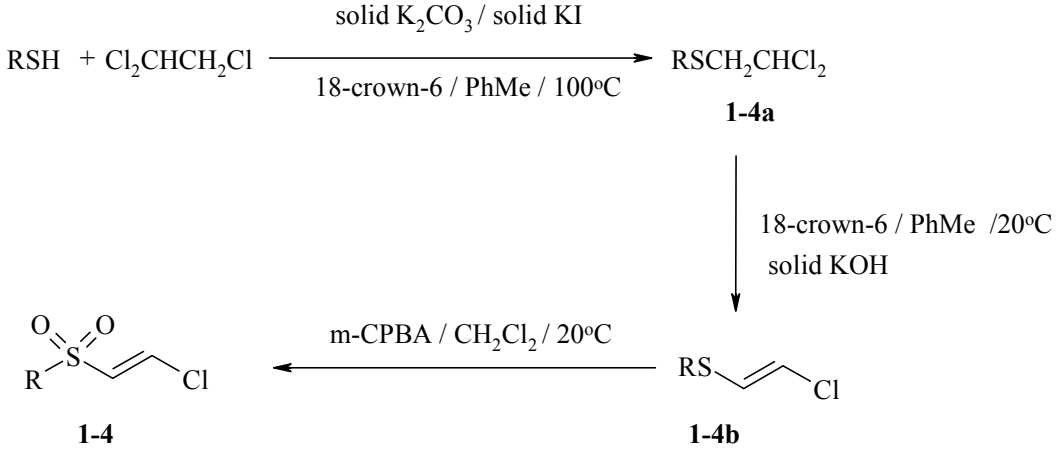
Functionalized unsaturated sulfur containing compounds have been investigated as intermediates in organic synthesis [1-3]. Among them 2-chlorovinylsulfones have been studied extensively [4-6]. 2-Chlorovinylsulfones are typically prepared by oxidation of 2-chlorovinyl sulfides with H_2O_2 in AcOH [7], m-chloroperbenzoic acid (m-CPBA) in CH_2Cl_2 [8] or by elimination of HCl from 2,2-dichlorovinylsulfones in the presence of Et_3N [9]. Some works were dedicated to conjugate addition-elimination of N- or S-nucleophiles to 2-chlorovinylsulfones. Thus, interaction of 2-chlorovinylsulfones with mercaptanes in the system $NaHCO_3$ / EtOH [10] or with sodium salts of mercaptanes [11] leads to addition products. In some cases triethylamine in diethylether was excellent system for the conjugate addition of mercaptanes to 2-chlorovinylsulfones (for example, 2-chlorovinyl-trifuoromethylsulfone [12]). 2-Chlorovinylsulfones react also with nitrogen nucleophiles forming addition products too [13-15]. However, stereoselectivity in most of above mentioned articles was low or not investigated at all. Beside this conjugate addition of N-, S- and Se-nucleophiles to 2-chlorovinylsulfones under phase transfer catalysis conditions hasn't been investigated till now.

Stereoselective conjugate addition of N-, S- and Se-nucleophiles to (*E*)-2-chlorovinylsulfones in good preparative yields is the problem that is solved in the present report. Beside this cytotoxicity of simple aryl and hetaryl substituted 2-chlorovinylsulfones hasn't been

investigated till now. Therefore the second aim is investigation of toxicity and cytotoxicity of (*E*)-2-chlorovinylsulfones and (*E)*-2-aziridinylvinylsulfones.

RESULTS AND DISCUSSION

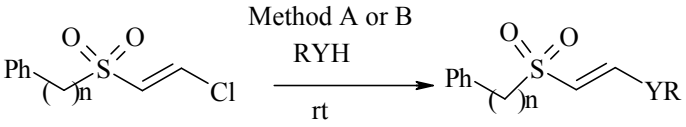
We obtained *E*-2-chlorovinylsulfones **1-4** by oxidation of an intermediate 2-chlorovinylsulfides **1-4b** with m-CPBA, prepared from corresponding thiols in the system $ClCH_2CHCl_2$ / K_2CO_3 (then KOH) / KI / 18-crown-6 / PhMe.



1-4 **1-4b**

R = Ph (**1**); R = $PhCH_2$ (**2**); R = 2-benzothiazolyl (**3**); R = 2-pyridyl (**4**)

We have developed novel PTC method for the *E*-stereoselective addition of N-, S- and Se-nucleophiles to (*E*)-2-chlorovinylthioarenes **1**, **2**. Experiments show, that catalytic system solid Cs_2CO_3 / 18-crown-6 / toluene was the best for the *E*-stereoselective synthesis of products **5-14** (Method B) (see Table 1). Under these conditions desired products were isolated in 6-91% yields. The synthesis of desired products **5-14** was carried out using triethylamine catalyzed synthesis too (Method A, see Experimental). Using above method alkenes **5-14** were isolated in 27-55% yields (see Tables 1 and 2). Thus, PTC method for the conjugate addition of N-nucleophiles to 2-chlorovinylsulfones in the most of the cases was more efficient than classical triethylamine catalyzed method.



1,2 **5-14**

n = 0,1; Y = NR', S, Se

Method A: Et_3N / PhMe

Method B: Cs_2CO_3 / 18-crown-6 / PhMe

The crystal structure of the furan derivative **11** was established by X-ray structure analysis. The molecular structure of sulfone **11** with atomic numbering scheme is presented in Fig. 1. The molecule consists of three planar fragments – phenyl ring, furan cycle and CH_2-S-CH=CH fragment. The values of C(2)-C(6)-S(7)-C(8) and C(8)-C(9)-C(10)-C(13) torsion angles

are equal 62.9(4) and −96.7(4)º, respectively. The structure has been deposited with the Cambridge Crystallographic Data Centre (deposition number is CCDC 721533).

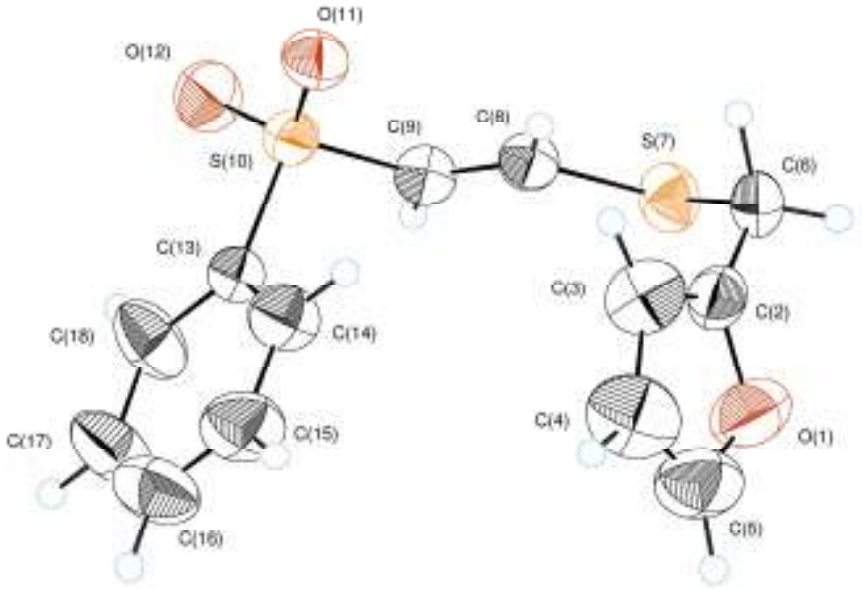
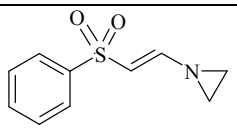
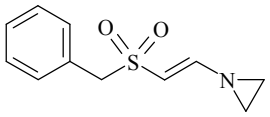
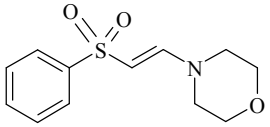
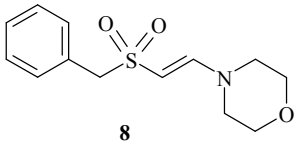
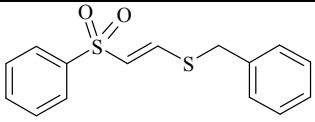
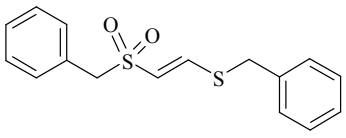
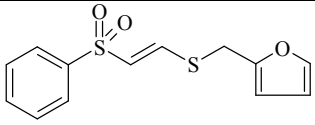
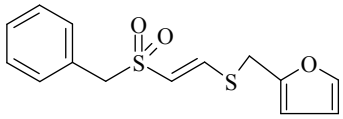
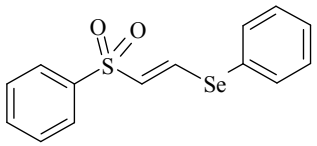
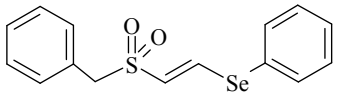


Fig. 1. Molecular structure of compound **11**.

Table 1. Conjugate addition of N-, S and Se-nucleophiles to 2-chlorovinylsulfones 1 and 2 (methods A and B, see Experimental)

Starting sulfone	Product	Method A		Method B	
		Reaction time, h	Yield, %	Reaction time, h	Yield, %
1	 **5**	24	38	40	60
2	 **6**	24	36	64	18
1	 **7**	24	36	20	91
2	 **8**	24	45	20	80
1	 **9**	28	38	63	20

2	 **10**	24	55	15	6
1	 **11**	36	27	70	13
2	 **12**	38	34	65	22
1	 **13**	24	51	24	20
2	 **14**	24	28	24	27

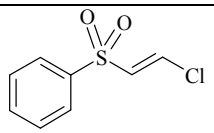
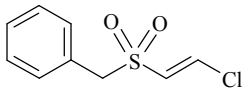
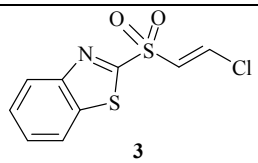
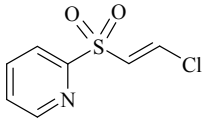
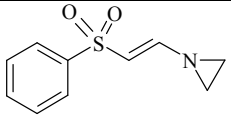
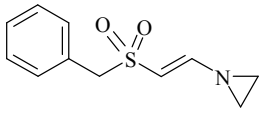
Cytotoxic activity of (*E*)-2-chlorovinylsulfones **1-4** and (*E)*-2-aziridinylvinylsulfones **5** and **6** was tested *in vitro* on the two monolayer tumor cell lines: MG-22A and HT-1080. Toxicity of chlorovinylsulfones **1-4** (LD_{50} 108-442 mg/kg) and 2-aziridinylvinylsulfones **5** and **6** (LD_{50} 628 and 782 mg/kg, harmful) was detected on the mouse normal fibroblasts. Preliminary analyses of the structure-activity relationship for the cytotoxic action clearly indicate the strong influence of chlorine or aziridine substituent on cytotoxic effects *in vitro*. Thus, chlorovinylsulfones **1, 2** and **4** exhibit very high cytotoxicity (IC_{50} values were ~1 μg/mL for the human fibrosarcoma HT–1080 cell and mouse hepatosarcoma MG-22A cell lines). Aziridine derivatives **5** and **6** were considerably less active on the above cancer cell lines (Table 3).

Table 2. MS, ^{1}H and ^{13}C NMR spectroscopic data of products 5 – 14.

Compound	MS, m/z (I, %)	^{1}H NMR, δ ppm	^{13}C NMR, δ ppm
5	209 (M^+, 7), 144 (40), 130 (42, 117 (78), 77 (100), 68 (50), 51 (92), 41 (82)	2.06 (s, 4H, aziridine protons), 5.81 and 7.50 (both d, 2H, J = 14 Hz, CH=CH), 7.49-7.55 and 7.84-7.88 (both m, 5H, Ph)	31.00 (aziridine), 110.02, 127.05, 131.10, 133.50, 136.30, 152.98
6	223 (M^+, 3), 158 (7), 132 (31), 91 (100), 65 (15)	1.98 (s, 4H, aziridine protons), 4.21 (m, 2H, CH_2), 5.57 and 7.11 (both d, 2H, J = 14 Hz, CH=CH), 7.36 (m, 5H, Ph)	27.59 (aziridine), 28.54 ($\underline{C}H_2$), 109.98, 126.41, 128.67, 128.98, 130.55, 130.87

7	253 (M^+, 29), 210 (20), 130 (27), 112 (88), 82 (100), 55 (45), 42 (42)	3.19 and 3.69 (both t, 8H, J = 5Hz, CH_2CH_2), 5.05 and 7.25 (both d, J =12 Hz, CH=CH), 7.39-7.49 and 7.83-7.86 (both m, 5H, Ph)	56.17 and 66.91 (morpholine), 110.18, 125.32, 126.43, 126.42 129.07, 132.00
8	267 (M^+, 10), 203 (8), 176 (100), 128 (15), 111 (17), 91 (72), 65 (25), 55 (15), 41 (20)	3.08 and 3.63 (both t, 8H, J = 5Hz, CH_2CH_2), 4.17 (s, 2H, CH_2), 4.78 and 6.75 (both d, J =12 Hz, CH=CH), 7.34 (m, 5H, Ph)	52.10 and 66.64 (morpholine), 63.22 ($\underline{C}H_2$), 109.89, 125.04, 128.20, 128.31, 130.51, 130.83
9	290 (M^+, <1), 148 (25), 91 (100), 65 (9)	3.99 (s, 2H, CH_2), 6.18 and 7.54 (both d, 2H, J =14 Hz, CH=CH), 7.50-7.59 and 7.69-7.83 (both m, 10H, Ph)	36.55 ($\underline{C}H_2$), 109.59, 122.00, 126.85, 126.71 128.22, 128.37, 128.81, 128.95, 132.66, 134.12
10	304 (M^+, <1), 214 (7), 148 (14), 91 (100), 65 (11)	3.94 and 4.14 (both s, 4H, CH_2), 5.97 and 7.18 (both d, 2H, J =14 Hz, CH=CH), 7.16-7.40 (m, 10H, Ph)	36.77 ($S\underline{C}H_2$), 61.96, 110.01, 128.05,128.27, 128.97, 128.64, 128.97, 130.82, 130.83, 131.13, 134.66
11	139 (M^+-$PhSO_2$, 23), 81 (100), 53 (11)	4.01 (s, 2H, CH_2), 6.27 and 7.73 (both d, 2H, J = 14 Hz, CH=CH), 6.24, 6.29 and 7.33 (all m, 3H, furan ring protons), 7.51-7.59 and 7.82-7.85 (both m, 5H, Ph)	25.94 ($\underline{C}H_2$), 108.37, 109.99, 110.66, 125.39, 127.32, 129.24, 133.41, 138.11, 141.20, 149.97
12	139 (M^+-$PhCH_2SO_2$, 25), 91 ($PhCH_2$, 70), 81 (100), 65 (10), 53 (12)	3.95 and 4.19 (both s, CH_2), 6.09 and 7.40 (both d, 2H, J = 16 Hz, CH=CH), 6.21 and 6.34 (both m, 2H, furan ring protons), 7.25-7.37 (m, 6H, Ph and furan ring proton)	29.15 ($\underline{C}H_2$), 61.91 ($\underline{C}H_2$), 108.88, 110.76, 118.97, 128.23, 128.72, 128.78, 130.85, 142.87, 147.26, 148.41
13	324 (M^+, 30), 234 (7), 182 (56), 157 (61), 125 (9), 102 (23), 77 (100), 51 (47)	6.19 and 8.21 (both d, 2H, J = 16 Hz, CH=CH), 7.37-7.60 and 7.80-7.84 (both m, 10H, Ph)	125.35, 127.13, 127.38, 129.20, 129.59, 130.03, 130.81, 133.14, 135.10, 140.63
14	338 (M^+, 5), 248 (7), 157 (15), 91 (100), 65 (16)	4.14 (s, 2H, CH_2), 5.92 and 7.90 (both d, 2H, J = 16 Hz, CH=CH), 7.29-7.49 (m, 10H, Ph)	61.65 ($\underline{C}H_2$), 123.65 124.97, 128.10 128.67, 129.65, 130.00, 130.78, 135.28, 145.96

Table 3. ***In vitro*** **cell cytotoxicity (IC_{50} (μg/ml) and intracellular NO-generation (nmolx10^2/200 μl, NO 100%CV, concentration 100 μg/ml) of compounds 1-6.**

Compound	3T3, LD_{50}, mg/kg	HT-1080			MG-22A		
		IC_{50}, CV	IC_{50}, MTT	NO 100%CV	IC_{50}, CV	IC_{50}, MTT	NO 100%CV
 **1**	162	<1	<1	150	<1	<1	75
 **2**	108	<1	<1	250	1	1	250
 **3**	442	10	10	60	15	27	100
 **4**	265	2	1	60	1	1	18
 **5**	628	17	13	67	20	14	57
 **6**	782	28	17	75	26	28	33

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Varian 200 Mercury spectrometer using $CDCl_3$ as a solvent and HMDSO as the internal standard. Mass spectra were registered on a GC-MS HP 6890 (70 eV) apparatus using a glass column packed with 5% OV-101 / Chromosorb W-HP (80-100 mesh, 1.2 m x 3 mm). Thiols, phenylselenide and morpholine, 18-crown-6 (Acros) were used without purification. Triethylamine and toluene were distilled over CaH_2 prior using. 2-Chlorovinylsulfones **1** [16], **2** [16, 17], **3** [18] and **4** [18] were prepared as described in articles.

Typical procedure for the synthesis of (*E*)-2-chlorovinylsulfones 1-4. 1,1,2-Trichloroethane (4.1 ml, 44 mmol) was added under stirring to the mixture of thiol (20 mmol), K_2CO_3 (8.28 g, 60 mmol), KI (6.64 g, 40 mmol) and 18-crown-6 (528 mg, 2 mmol) in 25 ml of xylene. Reaction mixture was refluxed for 2 hours, cooled and filtered. The solvent was evaporated under reduced pressure to obtain 2-(2,2-dichloroethanesulfanyl)hetarenes **1-4a**. Finely powdered KOH (2.24 g, 20 mmol) in 25 ml of toluene were added to reaction mixtures containing 2-(2,2-dichloroethanesulfanyl)hetarenes **1-4a**. Reaction mixture was stirred 45 minutes (GC-MS control) at room temperature, filtered and evaporated. The residue was purified by column

chromatography using hexane : toluene (2 : 1) as eluent to obtain intermediate 2-chlorovinylsulfides **1-4b**. *m*-Chloroperoxybenzoic acid (15.35 g, 88.9 mmol) was added portionwise under stirring to the solution of (*E*)-2-chlorovinylsulfides **1-4b** (29.8 mmol) in 50 ml of dry dichloromethane. Reaction mixture was stirred overnight at room temperature and filtered. The filtrate was concentrated at reduced pressure. The residue was purified by column chromatography using hexane : toluene (2 : 1) as eluent. The properties of obtained 2-chlorovinylsulfones **1,** [16] **2** [16], **3** [18] and **4** [18] were described in articles.

General method of synthesis for the conjugate addition of N-, S- and S-nucleophiles to 2-chlorovinylsulfones 1 and 2 in the presence of triethylamine (Method A). N-, S- or Se-nucleophile (1.1 mmol) was added under stirring to the solution of 2-chlorovinylsulfone **1** or **2** (1 mmol) and triethylamine (1.1 mmol) in 3 ml of dry toluene. Reaction mixture was stirred at room temperature (TLC control). The solvent was evaporated under reduced pressure. The residue was purified by column chromatography using hexane: ethyl acetate in different mixtures as eluent. The reaction conditions and spectroscopical data see Tables 1 and 2.

General method of synthesis for the conjugate addition of N-, S- and S-nucleophiles to 2-chlorovinylsulfones 1 and 2 under PTC conditions (Method B). N-, S- or Se-nucleophile (0.9 mmol) was added under stirring to the mixture of 1-chlorovinylsulfones **1** or **2** (1 mmol), Cs_2CO_3 (0.65 g, 2 mmol), and 18-crown-6 (26 mg, 0.1 mmol) in 2 ml of dry toluene. Filtered and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography using hexane : ethyl acetate in different mixtures as eluent. The reaction conditions and spectroscopical data see Tables 1 and 2.

X-Ray crystallographic analysis

A single crystal diffractometer "Bruker-Nonius KappaCCD" (MoK_α-radiation, λ = 0.71073Å) was used for data collection. Crystals of the compound are orthorhombic, space group $P2_12_12_1$ (No 19). Lattice parameters are a = 8.5931(3), b = 9.9710(3), c = 15.5512(7) Å; V = 1332.45(9) Å^3, Z = 4, $F(000)$ = 584, μ = 0.396 mm^{-1}, D_{calc} = 1.398 g/cm^3, $2\theta_{max}$ = 55.0°. A total of 5816 reflection intensities were collected at room temperature using φ scan technique. The structure was solved using direct method [19]. For structure refinement, 1371 independent reflections with $|F|^2 > 3\sigma(I)$ were used. The structure refinement was carried out with the *maXus* complex of programs [20]. The final *R*-factor is 0.042.

In vitro cytotoxicity assay

Monolayer tumor cell lines – HT-1080 (human connective tissue fibrosarcoma), MG-22A (mouse hepatosarcoma), 3T3 (mouse Swiss Albino embryo fibroblasts) - were cultured in standard medium (Dulbecco`s modified Eagle`s medium; DMEM) without an indicator ("Sigma") and supplemented with 10% heat-inactivated fetal bovine serum ("Sigma"). Tumor cell lines were taken from the ATCC. After the ampoule had thawed, cells from one to four passages were used. Three concentrations of test compound: 1, 10 and 100 μg ml $^{-1}$ was used and was tested. About (2-5) x10^4 cells ml^{-1} (depending on the nature of the line) were placed in 96-well plates immediately after compounds were added to the wells; the volume of each plate was 200 μl. The control cells without test compounds were cultured on separate plate. The plates were incubated for 72h, 37^{o}C, 5% CO_2. The number of surviving cells was determined using tri(4-dimethylaminophenyl)methyl chloride (crystal violet: CV) or 3-(4,5-dimethylthiazol-2-yl)-

2,5-diphenyltetrazolinium bromide (MTT) [21,22]. The quantity on the control plate was taken in calculations for 100%. The concentration of NO was determined according to the Griess method (by NO_2 level in the culture medium). Sodium nitrite standard solution was used for the calibration curve. LD_{50} was tested according „Alternative Toxicological Methods" [23]. The program Graph Pad Prism® 3.0 was used for calculations (r□< 0.05.).

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