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SYNTHESIS AND CYTOTOXICITY OF DERIVATIVES OF DI(3-INDOLYL) SELENIDE

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A method has been developed for the synthesis of di(3-indolyl) selenides. From indole and SeO_2. N-Alkyl derivatives of di(3-indolyl) selenide have been obtained in the two-phase system alkyl halide–solid K_2CO_3 (or KOH)–18-crown-6–toluene. It was discovered that N-unsubstituted di(3-indolyl) selenides possess high cytotoxicity on HT-1080 and MG-22A tumor cell lines.

Keywords: indole, selenides, phase-transfer catalysis, cytotoxicity.

Selenium-containing indoles are used in organic synthesis as intermediates for radical cyclization [1,2]. These indoles are used as biologically active compounds. It was shown recently that amides of 2,2'-diselenobis(1H-indoles) actively inhibit tyrosine kinase [3,4]. On the other hand, derivatives of benzoseleno[4,3-*b*]indole [5] are carcinogens [6]. Among recent studies it is necessary to note investigations on routes of synthesis and properties of 1,2,5-selenadiazolo[3,4-*e*]indoles and their [3,4-*f*] and [3,4-*g*] isomers [7].

The classical method of synthesizing 3,3'-diindolyl diselenides is based on the selenocyanation of indole with bis-seleno cyanide with subsequent treatment of the reaction mixture with KOH solution in methanol [8-11]. Some work has been devoted to the synthesis of indole selenides. Previously β-(3-indolyl)ethylselenoacetic acid was obtained from β-(3-indolyl)ethyl bromide and $(HO_2CCH_2Se)_2$ [12]. In addition 3,3'-diindolyl selenides were successfully synthesized by the reaction of indoles with SeO_2 in benzene [13]. Bisharmine selenides were obtained analogously [14]. The mass spectrometric investigation of indole selenides has been described in detail in [15]. It has also been shown that di(1-methyl-3-indolyl) selenide in the presence of Raney nickel gives biindolyl and indole.

Investigation of the cytotoxicity of derivatives of di(3-indolyl) selenides has not been carried out previously and is the aim of the present work.

Di(3-indolyl) selenides **3**, **4** were obtained from indole **1** and 2-methylindole **2** by the procedure reported in [13]. In the reaction of 2-methylindole with SeO_2 in boiling benzene after 2 h the author of [13] obtained di(2-methyl-3-indolyl) triselenide (16%) and di(2-methyl-3-indolyl) diselenide (22%). Under similar conditions (4 h in boiling benzene) we obtained di(2-methyl-3-indolyl) selenide (**4**) as the sole product, but in only 7% yield. The structure of selenide **4** was confirmed by data of elemental analysis, and ^1H, ^{13}C, and ^{77}Se NMR spectra. The ^{77}Se chemical shift for selenide **4** has a characteristic value (1287.29 ppm) for this class of compound.

Di(3-indolyl) selenide (**3**) in the system alkyl halide (R'X)–solid K_2CO_3 (or KOH)–18-crown-6–toluene forms the N-substituted selenides **5**-**8** in 28-38% yield (Tables 1-3). In the reaction of selenide **3** with $Br(CH_2)_5Br$ in the system solid K_2CO_3–18-crown-6–toluene the mono N-alkylated product **9** was obtained in 30% yield. The cyclic product is not formed under these conditions.

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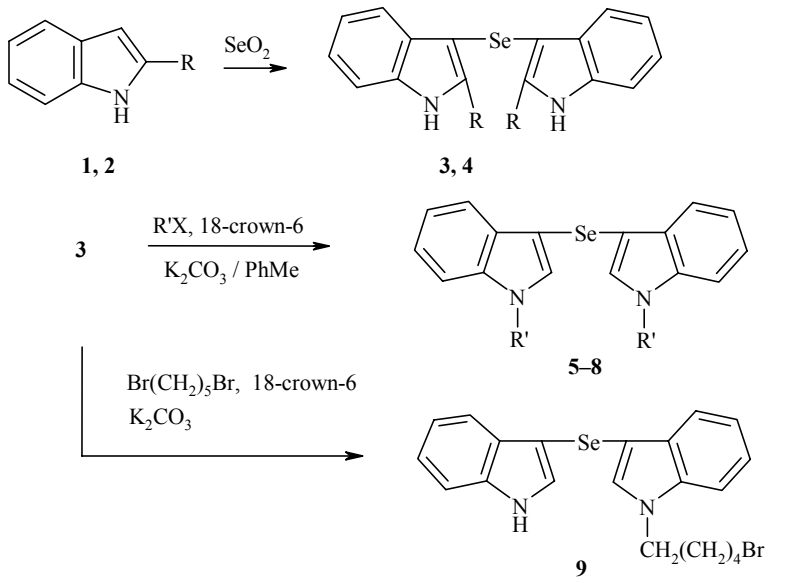

1, 3 R = H; **2, 4** R = Me; **5** R' = Me; **6** R' = $PhCH_2$; **7** R' = 2-$MeC_6H_4CH_2$; **8** R' = $Me_3Si(CH_2)_3$.
In the synthesis of **6, 7** X = Br; **5, 8** X = I

The biological activity of the obtained compounds was investigated on two tumor cell lines, *viz*. HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) (Table 4). Di(3-indolyl) selenide (**3**) possesses the greatest cytotoxic effect. This compound shows an IC_{50} of 1 μg/ml (CV test) for the fibrosarcoma and 2 μg/ml (CV and MTT tests) for the mouse hepatoma. The methyl-substituted selenide **4** also showed a similar activity. The N-alkyl derivatives of selenide **3** showed low cytotoxicity. The high level of NO generation by selenide **3** should be noted (450% in the HT-1080 line and 400% in the MG-22A line).

EXPERIMENTAL

The ^1H, ^{13}C, and ^{77}Se NMR spectra were recorded on a Varian 200 Mercury spectrometer (200, 50, and 39 MHz respectively) in $CDCl_3$ (in DMSO-d_6 for compounds **3** and **4**), internal standard was HMDS. For the ^{77}Se NMR spectra the internal standard was SeO_2 (δ 1275.5 ppm). Indole, 2-methylindole, selenium dioxide, methyl iodide, benzyl bromide, 2-methylbenzyl bromide, and 18-crown-6 (Acros) were used without further purification. 3-Iodopropyl(trimethyl)silane was obtained by the Grignard reaction [16,17]. Di(3-indolyl) selenide (**3**) was obtained from indole and SeO_2 in benzene as described in [13].

TABLE 1. Interphase Catalyzed Alkylation of Di(3-indolyl) Selenide **3**

R'X	Reaction time, h	Product	Empirical formula	Found, % / Calculated, %			mp, °C	Yield, %
				C	H	N		
MeI	7	**5**	$C_{18}H_{16}N_2Se$	63.46 / 3.71	4.74 / 4.75	8.10 / 8.25	160-162	38
$PhCH_2Br$	8	**6**	$C_{30}H_{24}N_2Se$	73.22 / 73.31	4.81 / 4.92	5.65 / 5.70	>300 (dec.)	28
2-$MeC_6H_4CH_2Br$	14	**7**	$C_{32}H_{28}N_2Se$	73.71 / 73.97	5.44 / 5.43	5.26 / 5.39	168-169	33
$Me_3Si(CH_2)_3Br$	10	**8**	$C_{28}H_{40}N_2SeSi_2$	61.04 / 62.30	7.38 / 7.46	4.84 / 5.18	69-70	28

TABLE 2. Data of ^{1}H and ^{13}C NMR Spectra for Selenides **3-8**

Selenide	^{1}H NMR, δ, ppm (*J*, Hz)	^{13}C NMR spectra, δ, ppm	
		Indole	R'
3	7.04, 7.33 and 7.66 (10H, all m, ring protons); 11.28 (2H, br. s, NH)	98.94, 111.75, 119.36, 19.44, 121.52, 129.40, 130.52, 136.19	—
4	2.59 (6H, s, CH_3); 6.96, 7.21 and 7.54 (8H, all m, ring protons); 11.24 (2H, br. s, NH)	18.26 (CH_3), 102.74, 115.88, 123.90, 124.37, 125.88, 135.76, 140.56, 144.47	—
5	3.66 (6H, s, CH_3); 7.13-7.22 and 7.81 (10H, m, ring protons)	99.55, 109.26, 119.79, 120.36, 121.91, 130.28, 133.64, 137.03	32.77 (CH_3)
6	5.20 (4H, s, CH_2); 7.0-7.3 and 7.77 (20H, m and m, ring protons)	99.98, 109.81, 120.02, 120.53, 122.12, 130.49, 132.98, 137.03	50.10 (CH_2), 126.79, 127.65, 128.76, 136.75 (all Ph)
7	2.22 (6H, s, CH_3); 5.18 (4H, s, CH_2); 7.13-7.24 and 7.75 (18H, m and m, ring protons)	99.82, 109.64, 119.98, 120.51, 122.04, 130.20, 132.68, 136.82	19.02 (CH_3), 48.15 (CH_2), 109.73, 126.64, 127.51, 127.89, 130.27, 135.74 (all Ph)
8	-0.05 (18H, s, $Si(CH_3)_3$); 0.44 (4H, m, $SiCH_2$); 1.75 (4H, m, $CH_2C\underline{H_2}CH_2$); 3.99 (4H, t, *J* = 7.2, NCH_2); 7.09-7.28 and 7.84 (10H, m and m, ring protons)	99.19, 109.45, 119.64, 120.45, 121.72, 130.36, 132.64, 136.40	-1.81 ($SiMe_3$), 13.91 ($SiCH_2$), 24.89 ($CH_2C\underline{H_2}CH_2$), 49.65 (NCH_2)

Di(2-methyl-3-indolyl) Selenide (4). A suspension of 2-methylindole **2** (7.86 g, 30 mmol) and SeO_2 (4.40 g, 39.6 mmol) in benzene (90 ml) was refluxed for 5 h. The reaction mixture was filtered, and the filtrate was evaporated to 20 ml on a rotary evaporator. Hexane was added to turbidity of the reaction mixture. The product was twice recrystallized at 4°C from benzene–hexane. The yield of compound **4** was 0.70 g (7%). ^{77}Se NMR spectrum, δ, ppm: 1287.29.

TABLE 3. Mass Spectra of Selenides **4-8**

Selenide	*m/z* (I_{rel}, %)*
4	340 $[M]^+$ (37), 338 (17), 260 (95), 245 (16), 243 (17), 217 (13), 209 (29), 207 (13), 131 (95), 130 (100), 122 (16), 117 (9), 115 (14), 103 (60), 102 (35), 89 (25), 78 (50), 77 (70), 65 (32), 63 (37)
5	340 $[M]^+$ (13), 338 (7), 260 (100), 245 (30), 130 (38), 122 (10), 89 (15), 77 (15), 71 (15), 69 (15), 63 (10), 61 (14)
6	492 $[M]^+$ (20), 412 (100), 321 (95), 204 (25), 177 (8), 130 (11), 102 (11), 97 (12), 91 (95), 89 (35), 77 (24), 71 (17), 69 (25), 65 (72)
7	520 $[M]^+$ (19), 440 (100), 414 (3), 367 (10), 335 (92), 231 (19), 221 (52), 218 (20), 117 (12), 106 (77), 105 (90), 103 (68), 91 (12), 89 (15), 79 (75), 77 (70), 69 (11), 65 (14), 63 (13)
8	540 $[M]^+$ (8), 462 (16), 461 (45), 460 (100), 359 (17), 231 (17), 157 (20), 130 (54), 97 (12), 95 (19), 83 (15), 75 (15), 73 (75), 71 (16), 69 (19)

* Ions having *m/z* >60 and I_{rel} >10% are shown. For selenium-containing fragments ions with the ^{80}Se isotope are given.

TABLE 4. Cytotoxic Activity of Selenides **3-8** *in vitro*

Compound	Cell lines					
	HT-1080			MG-22A		
	IC_{50}* CV*[2]	IC_{50}, MTT*[3]	NO100% CV*[4]	IC_{50}* CV	IC_{50}* MTT	NO 100% CV
3	1	2	450	2	2	400
4	4	12	300	2	3	250
5	23	26	53	*[5]	*[5]	10
6	*[5]	*[5]	6	*[5]	*[5]	218
7	*[5]	*[5]	12	*[5]	*[5]	13
8	*[5]	*[5]	18	*[5]	*[5]	20

* Concentration μg/ml causing death of 50% cells.
*[2] Staining with Crystal Violet.
*[3] Staining with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide.
*[4] NO concentration (%) (CV staining).
*[5] No cytotoxic activity.

General Procedure for Interphase Catalysed Alkylation of Di(3-indolyl) Selenide (3)*. The appropriate alkyl halide (2.2 mmol) was added to a suspension of compound **3** (0.311 g, 1 mmol), 18-crown-6 (0.026 g, 0.1 mmol), and powdered KOH (0.224 g, 4 mmol) [in the synthesis of compound **8** powdered K_2CO_3 (0.552 g, 4 mmol) was used] in toluene (10 ml). The reaction mixture was stirred at room temperature (100°C for compound **8**) for 8-14 h, filtered, and the filtrate was evaporated on a rotary evaporator. The residue was purified by column chromatography (eluent was hexane–ethyl acetate in various concentrations), and compounds **5-8** were obtained (Tables 1-3).

1-(5-Bromopentyl)-3-[(3-indolyl)seleno]indole (9). 1,5-Dibromopentane (0.14 ml, 1 mmol) was added to a suspension of compound **3** (0.311 g, 1 mmol), 18-crown-6 (0.026 g, 0.1 mmol), and powdered K_2CO_3 (0.414 g, 4 mmol) in toluene (10 ml). The reaction mixture was stirred at 100°C for 40 h, filtered, and the filtrate evaporated on a rotary evaporator. The residue was purified by column chromatography (eluent hexane–ethyl acetate, 1:0.3), and compound **9** (0.14 g, 30%) was obtained having mp 138-142°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.2-1.8 [8H, m, $Br(CH_2)_4$]; 3.99 (2H, t, *J* = 5.6, NCH_2); 6.18 (1H, s, NH); 7.20-7.33 and 7.95 (10H, m, ring protons).

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