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CYTOTOXIC DI(8-QUINOLYL) DISULFIDES

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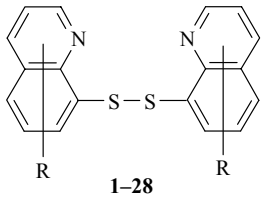
It has been shown that the nature of the substituent and its position in the quinoline ring markedly affects the antitumor activity and toxicity of di(8-quinolyl) disulfides. The greatest cytotoxicity in the series of methyl derivatives was shown by the 7-, 6-, and 3- isomers towards HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) tumor cells while the 2-methyl derivatives generally have no effect on these cells. High cytotoxicity was also shown (LC_{50} <1 μg/ml) by other 7- substituted compounds (Cl, PhO, PhS) but they also appear to be highly toxic towards normal NIH 3Y3 mouse embryonic fibroblasts. A similar trend was observed in the series of 5- substituted compounds (NH_2, Cl, OMe, NO_2) which were highly active towards tumor cells but were toxic to normal cells. The best selectivity was found for the 6- substituted quinolines, the 6-methoxy derivative at low concentration brought about the death of tumor cells but appeared much less toxic towards normal fibroblasts (LC_{50} 100 μg/ml with a corresponding LD_{50} of 874 mg/kg).

Keywords: di(8-quinolyl) disulfides, toxicity, cytotoxicity.

We have previously shown that di(8-quinolyl) disulfide **1** has high cytotoxicity (LC_{50} 0.3-0.4 μg/ml) towards HT-1080 tumor cells (human fibrosarcoma) and MG-22A (mouse hepatoma) [1]. However, while this disulfide is highly active towards tumor cells it is toxic towards normal NIH 3T3 mouse embryonic fibroblasts (LC_{50} 0.7 μg/ml) [1].

With the aim of decreasing the toxicity and increasing the selectivity we have prepared a series of disulfides containing one (**2-25**) or two (**26-28**) electron donor or electron acceptor substituents both in the pyridine and in the benzene part of the quinoline ring and we have studied the effect of the nature and position of the substituent on their cytotoxicity (LC_{50}) in two lines of tumor cells (HT-1080 and MG-22A) as well as to normal NIH 3T3 fibroblasts which served as a measure of the toxicity of the compound (alternative method of determining LD_{50} see [2]).



1–28

1 R = H, **2** R = 2-Me, **3** R = 3-Me, **4** R = 4-Me, **5** R = 5-Me, **6** R = 6-Me, **7** R = 7-Me, **8** R = 2-Ph, **9** R = 4-Ph, **10** R = 5-Ph, **11** R = 6-Ph, **12** R = 4-Cl, **13** R = 5-Cl, **14** R = 7-Cl, **15** R = 2-MeO, **16** R = 5-MeO, **17** R = 6-MeO, **18** R = 4-PhO, **19** R = 5-PhO, **20** R = 7-PhO, **21** R = 5-PhS, **22** R = 7-PhS, **23** R = 5-SO_3H, **24** R = 5-NO_2, **25** R = 5-NH_2, **26** R = 2,4-Me_2, **27** R = 2,7-Me_2, **28** R = 5,7-Cl_2

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TABLE 1. Cytotoxicity of Di(5-R-8-quinolyl) disulfides*

Compound	Empirical formula	Found, % / Calculated, %			mp, °C	Yield, %
		C	H	N		
4a	$C_{16}H_{15}N_3O_4$	61.48 / 61.34	4.95 / 4.83	13.33 / 13.41	182 (dec.)	72
4b	$C_{17}H_{17}N_3O_4$	62.30 / 62.38	5.32 / 5.23	12.71 / 12.84	190 (dec.)	74
4c	$C_{18}H_{19}N_3O_4$	63.46 / 63.33	5.73 / 5.61	12.44 / 12.31	193 (dec.)	77
6a	$C_{14}H_9N_3O_3$	62.82 / 62.92	3.27 / 3.39	15.64 / 15.72	> 335	98
6b	$C_{15}H_{11}N_3O_3$	64.13 / 64.05	3.99 / 3.94	14.85 / 14.94	> 335	97
6c	$C_{16}H_{13}N_3O_3$	65.01 / 65.08	4.38 / 4.44	14.30 / 14.23	> 335	98

* CV = crystal-violet; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; NR = neutral-red.

The introduction of a substituent in position 5 of the quinoline ring (one of the remotest from the nitrogen and sulfur atoms) decreases the toxicity of all of the disulfides **5, 10, 13, 16, 19, 21, 23-25** when compared with the toxicity of the unsaturated disulfide **1** but to a different extent depending on the nature of the substituent (Table 1). The 5-methyl derivative **5** was found to be the least toxic (LC_{50} 750 μg/ml). The 5-phenyl (**10**) and 5-phenylthio (**21**) derivatives also showed low toxicity (LC_{50} 240 and 184 μg/ml respectively). The oxygen containing analog of the latter (the 5-phenoxy derivative **19**) proved to be several times more toxic. The greatest toxicity amongst the 5- substituted quinolines were the amino, methoxy, chloro, and nitro derivatives **25, 16, 13, 24** respectively (LC_{50} 4-8 μg/ml). These compounds show the greatest toxicity towards tumor cells (in the majority of cases the LC_{50} was less than 1 μg/ml). In terms of their effect on cytotoxicity (Table 1) these compounds fall in almost the same sequence as their toxicity:

$$Ph < PhS < SO_3H < Me < PhO < NO_2 < MeO < Cl, NH_2$$

The 5-methyl derivative **5** proved to be an exception having the least toxicity in this series of compounds but with reasonable cytotoxicity towards MG-22A cancer cells (LC_{50} 3-8 μg/ml) and NT-1080 (LC_{50} 6 μg/ml. MTT test). Hence we have studied the effect of the position of a methyl group in the quinoline ring on the antitumor activity and toxicity of compounds **2-7** (Table 2).

TABLE 2. Cytotoxicity of Di(methyl-8-quinolyl) disulfides

Compound*	LC_{50}, μr/ml					LD_{50}, mg/kg
	HT-1080		MG-22A		3T3	
	CV	MTT	CV	MTT	NR	
2	*[2]	*[2]	*[2]	*[2]	*[2]	>2000
3	3	1	<1	<1	38	557
4	16	5	2	2	15	383
5	68	6	3	8	750	2018
6	2	<1	<1	<1	14	348
7	<1	<1	<1	<1	5	209

* Compound number corresponds to the position of the methyl group in the quinoline ring.
*[2] Cytotoxic effect absent.

The cytotoxicity of the isomeric methyl quinolines towards HT-1080 and MG-22A tumor cells falls in the following sequence:

7-Me > 6-Me > 3-Me > 4-Me > 5-Me >> 2-Me

Their toxicity falls in almost the same sequence. The least toxic is the 2-methyl derivative **2** (LD_{50} > 2000 mg/kg) which proved completely inactive towards tumor cells.

The 2-phenyl derivative **8** also does not show antitumor activity. Of the methyl quinoline derivatives the 7-methyl derivative **7** shows the highest activity (LC_{50} < 1μg/ml).

Other 7-substituted quinolines show the same high activity, *viz* the chloro (**14**), phenoxy (**20**), and phenylthio (**22**) di(8-quinolyl) disulfides (Table 3). However, in all of these examples the high cytotoxicity towards tumor cells is linked to a high toxicity towards normal cells.

It was of interest to study the properties of compounds with introduction of two methyl groups into positions where in one the monosubstituted compound is of low and in the other of high activity. In the case of 2,4-dimethyl derivative **26** the introduction of the second methyl group at position 2 completely suppresses the activity of the 4-methyl derivative **4** (LC_{50} 2 μg/ml towards MG-22A cells). In contrast, the introduction of a second methyl group into the molecule of the highly active 7-monosubstituted derivative **7** virtually does not decrease its activity and the LC_{50} of the 2,7-dimethyl derivative **27** is also less than 1 μg/ml in the majority of tests. In the examples of the chloro derivatives the cytotoxicity of the 5,7-disubstituted **28** is somewhat less than the 5- and 7-mono chloro derivatives **13** and **14** (Table 3).

Amongst the highly active methyl derivatives the 6-substituted isomer was somewhat less toxic. The 5-substituted isomer was significantly less (but with good activity) and the methoxy derivative **15** (LC_{50} 2μg/ml for MG-22A) was the most active amongst all of the 2-substituted series. Hence we measured the cytotoxicity of the compounds with a methoxy group in positions 5 and 6. Both isomers **16** and **17** showed high cytotoxicity, especially towards MG-22A cells (LC_{50} ~ 1 μg/ml) but the 6-methoxy derivative **17** was 25 times less toxic towards normal cells (LC_{50} ~ 100 μg/ml) than the 5-isomer **16**.

Hence we have demonstrated the marked effect of the nature and position of a substituent in the quinoline ring on the antitumor activity and toxicity of substituted di(8-quinolyl)disulfides.

TABLE 3. Cytotoxicity of Di(R-8-quinolyl) disulfides

Compound	LC_{50}, μg/ml					LD_{50}, mg/kg
	HT-1080		MG-22A		3T3	
	CV	MTT	CV	MTT	NR	
8	*	*	*	*	*	>2738
9	19	6	22	20	16	453
11	2	<1	10	36	16	453
12	16	8	4	9	60	718
14	<1	<1	<1	<1	<0.3	<25
15	19	6	2	2	394	1596
17	10	<1	<1	1	100	874
18	22	18	15	32	32	655
20	1	<1	<1	<1	1.4	151
22	2	<1	1	2	5	268
26	*	*	*	*	1000	2419
27	2	<1	<1	<1	1.5	151
28	12	4	3	4	6	265

* Cytotoxic effect absent.

A group of highly active compounds was revealed (with the substituents 7-Me, 7-Cl, 7-PhO, 2,7-Me_2, 6-MeO, 6-Me, 5-NH_2, 5-OMe, 5-Cl, and 3-Me) with an LC_{50} < 1 μg/ml in the majority of tests. A rather high sensitivity of MG-22A towards the action of these compounds was found. Another group of compounds are either inactive (2-Me, 2-Ph, 2,4-Me_2) or show low cytotoxicity (5-Ph, 5-PhS, 5-SO_3H, 4-PhO). In their toxicity relationship a group of low toxicity compounds was found (2-Ph, 2-Me, 2,4-Me_2, 2-MeO, 5-Me, 5-Ph, 5-PhS) for which LD_{50} > 1400 mg/kg and a group of compounds (7-Cl, 7-PhO, 2,7-Me_2, 7-Me, 7-PhS, 5-NH_2, 5-OMe) having a marked toxicity (LC_{50} < 5 μg/ml) towards normal mouse 3T3 fibroblast cells.

By varying the substituent and position in the quinoline ring compounds with good selectivity of action can be developed. Hence of the group of highly active compounds the introduction of a methoxy group at position 6 gave the di(6-methoxy-8-quinolyl) disulfide **17** with LC_{50} ~ 1 μg/ml for a series of tumor cells and significantly less (100 μg/ml) for normal cells (corresponding LD_{50} 874 mg/kg).

EXPERIMENTAL

Di(8-quinolyl)disulfides were synthesized by the following methods: **1** [3], **2** [4], **3** [5], **4** [6], **5** [7], **6** [8], **7** [9], **8** [10], **9** [11], **10,11** [12], **12** [13], **13** [14], **14** [15], **15** [16], **16** [17], **17** [18], **18** [19], **19,20** [20], **21,22** [21], **23** [22], **24** [23], **25** [24], **26** [25], **27** [26], and **28** [27].

Cytotoxicity of compounds 1-28. *in vitro* relative to monolayers of HT-1080 tumor cells (human fibrosarcoma), MG-22A (mouse hepatoma), and NIH-3T3 normal cells (embryonic mouse fibroblasts) were measured in 96 well plastic plates using the dyes CV, MTT, and NR according to methods [28, 29] certified by us earlier [1, 30-32]. The concentrations of compounds causing the death of 50% of the cells (LC_{50}, μg/ml) are given in Tables 1-3.

The acute toxicities (LD_{50}, mg/kg) were calculated by methods [2, 33] using data for the 3T3 cell culture.

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