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Cytotoxicity of new polyfluorinated 1,4-naphtoquinones with diverse substituents in the quinone moiety

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ABSTRACT

Fluorinated derivatives of 1,4-naphthoquinones are highly potent inhibitors of Cdc25A and Cdc25B phosphatases and growth of tumor cells. Eight new derivatives of polyfluoro-1,4-naphthoquinone were synthesized and their cytotoxicity in human myeloma, human mammary adenocarcinoma, mouse fibroblasts and primary mouse fibroblast cells as well as their mutagenic and antioxidant properties in a *Salmonella* tester strain were studied. The efficiency of suppressing the growth of two lines of tumor cells decreased in the order: 2-(2-hydroxy-ethylamino)-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (**1**), 2,3-dimethoxy-5,6,7,8-tetrafluoro-1,4-naphthoquinone (**2**), 2-[2-hydroxyethyl(methyl)amino]-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (**3**), 2-morpholino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (**4**), 2-[bis-(2-hydroxy-ethyl)amino]-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (**5**), 2-[(2-hydroxy)ethylsulfanyl)]-5,6,7,8-tetrafluoro-1,4-naphthoquinone (**6**), 2-methoxy-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (**7**), and 1,4-dioxo-3-(1-pyridinio)-1,4-dihydro-5,6,7,8-tetrafluoronaphthalene-2-olate (**8**). Taking into account these data together with the better cytotoxic effect against cancer cells as compared with normal mammalian cells, protecting of bacterial cells from spontaneous and H_2O_2-dependent mutagenesis, and lower general toxicity of the compounds towards different cells, one can propose that compounds **3–5** may be considered as useful potential inhibitors of growth of tumor cells.

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1. Introduction

Members of the Cdc25 family of phosphatases remove phosphates from cyclin-dependent kinases (Cdk) and thus activate cyclin–Cdk complexes, which control cell cycle progression.[1,2] Cdc25A and Cdc25B are overexpressed in a number of tumors of various origins, frequently showing correlations with higher-grade or more aggressive tumors and poor prognosis.[3] The putative involvement of the Cdc25 phosphatases in tumorigenesis makes these cell cycle regulators potential targets for cancer therapy.[4,5] A majority of the known small molecule Cdc25 inhibitors are quinones or quinone-type compounds;[6,7] NSC 95397 (2,3-bis[(2-hydroxyethyl)thio]-1,4-naphthoquinone) from the National Cancer Institute Library is known as the most potent Cdc25 inhibitor.[8] *p*-Naphthoquinones and 7-aminoquinoline-5,8-quinones were used for the synthesis of many potential inhibitors of Cdc25 phosphatases[8] including effective inhibitors NSC 663284.[9] Cpd5, or 2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone effectively inhibited Cdc25 phosphatases.[10]

Although most quinones have been reported to inhibit Cdc25 by sulfhydryl arylation at the quinone moiety, due to redox properties they can also generate toxic oxygen species,[11] which may cause toxicity to normal tissues and thus reduce their therapeutic utility. One strategy for overcoming the intrinsic toxicity of quinones might be to use derivatives that are more stable in their reduced state and thus are less likely to initiate formation of radicals and indiscriminately damage cells. Interestingly, fluorinated derivatives of 1,4-naphthoquinones are less prone to form reactive oxygen species (ROS); among several substances, the most potent inhibitor of Cdc25A and Cdc25B phosphatases was fluorinated Cpd5 (F-Cpd5).[12–14] F-Cpd5 was calculated to have much higher reduction potential compared with Cpd5 and predicted not to generate ROS.[12] F-Cpd5 is three times more potent than Cpd5 in inhibiting Hep3B cell growth[12–14] and inhibits mitogen-induced DNA synthesis in normal rat hepatocytes 12-fold less than in Hep3B cells.[13]

Synthesis and analysis of new polyfluorinated naphthoquinones are directed at a search of new more effective inhibitors of tumor cell growth, which include the study of the efficiency of Cdc

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phosphatases inhibition and redox potential of the test compounds. It is known that all naphthoquinones including fluorinated ones can more or less efficiently inhibit Cdc25 phosphatases.[8–14] However depending on the structure of inhibitors of Cdc25 phosphatases but regardless of inhibition activity and redox potential, they might be polyfunctional compounds interacting with other cellular targets showing toxic, mutagenic or carcinogenic properties. Since all polyfluorinated naphthoquinones are inhibitors of Cdc25 phosphatases, only the data concerning toxic, mutagenic or carcinogenic properties could help to estimate a possibility of their application as antitumor drugs. Taking this into account, a measurement of their affinity for Cdc25 phosphatases and redox potential seems to be of secondary importance.

For rationale searching of new effective inhibitors of Cdc25 phosphatases with better inhibition of tumor cells growth but with minimal side effects, we have recently proposed to compare their cytotoxicity in human and mouse tumor cells and in primary mouse fibroblast cells in parallel with evaluation of their toxic, mutagenic, and antioxidant properties.[15,16] Interestingly, several new polyfluorinated *n*-alkylamino-, phenylamino-, and alkylthioderivatives of 1,4-naphthoquinone suppressed the growth of cancer cells at significantly lower concentrations than normal cells and antioxidant and mutagenic properties in the bacterial system significantly depend upon nature of the functional groups, only 3–4 of them possessing a highly promising combination of these properties.[15,16] Thus, it seems reasonable to suggest that this approach may be more rational for searching new inhibitors of Cdc25 phosphatases and tumor cell growth without side effects.

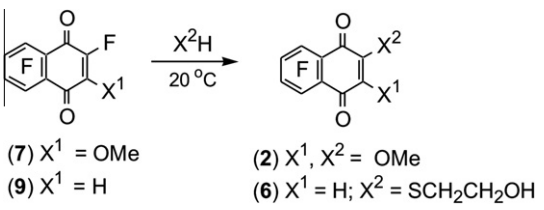
In this work, for eight new fluorinated derivatives of 1,4-naphthoquinone, containing diverse functional groups, cytotoxicity against cancer cells and ability to protect bacterial cells from spontaneous and H_2O_2-induced mutagenesis were compared.

2. Results and discussion

2.1. Chemistry

In this article we have study biological properties of eight new fluorinated derivatives of 1,4-naphthoquinone, the synthesis of six of which was described earlier. Quinones **1, 3**–**5**, **7**, and **8** were synthesized recently by amino- or methoxydefluorination of hexafluoro-1,4-naphthoquinone (**10**) with corresponding amines or MeOH.[17] Previously unknown compounds **2** and **6** were prepared via the reactions of 2-methoxy-3,5,6,7,8-pentafluoro-1,4-naphthoquinone or 2,5,6,7,8-pentafluoro-1,4-naphthoquinone[18] with MeOH or 2-mercaptoethanol, respectively (Scheme 1). Their structures were confirmed by ^{1}H and ^{19}F NMR spectra (in DMSO-d_6 or dry acetone-d_6), and elemental analysis.

In the ^{19}F NMR spectrum of **2** there are two signals at δ 23.9 and 17.3 ppm (vs hexafluorobenzene as an internal reference) belonging to the pairs of atoms F^5, F^8 and F^6, F^7, respectively, and slightly high-field shifted compared with quinone **10** (F^5, F^8 δ 25.5; F^6, F^7 δ 18.6 ppm) by an electron-releasing effect of the methoxy groups. The location and multiplicities of the F^5–F^8 signals of quinone **6**:



Scheme 1. Synthesis of compounds **2** and **6**. Reagents and conditions: for (**2**) X^2H = MeOH, Et_3N, 48 h; for (**6**) X^2H = $HSCH_2CH_2OH$, MeOH, 30 min.

δ/ppm 23.8 (dt, F^8, $J_{8,5}$, $J_{8,6}$ ~12 Hz, $J_{8,7}$ = 19.0 Hz), 22.4 (ddd, F^5, $J_{5,6}$ ~19 Hz, $J_{5,7}$ ~10 Hz, $J_{5,8}$ – 12.5 Hz), 16.7 (dt, F^6, $J_{6,5}$, $J_{6,7}$ ~19 Hz, $J_{6,8}$ = 11.2 Hz), 14.6 (dt, F^7, $J_{7,6}$, $J_{7,8}$ ~19 Hz, $J_{7,5}$ = 9.9 Hz) are close to those of 2-*n*-butylaminopentafluoro-1,4-naphthoquinone (**11**): δ/ppm 25.8 (dt, F^8, $J_{8,5}$, $J_{8,6}$ ~12 Hz, $J_{8,7}$ ~20 Hz), 24.2 (ddd, F^5, $J_{5,6}$ ~20 Hz, $J_{5,7}$ ~10 Hz, $J_{5,8}$ ~12 Hz), 19.8 (ddt, F^6, $J_{6,5}$, $J_{6,7}$ ~20 Hz, $J_{6,3}$ = 4.5 Hz), 15.0 (dt, F^7, $J_{7,5}$ ~10 Hz, $J_{7,6}$, $J_{7,8}$ ~20 Hz).[15] The obvious main difference between the ^{19}F NMR spectra of **6** and **11** is an absence of the F^3–F^6 spin coupling in the case of **6**.

In the ^{1}H spectrum of **2** a singlet of the methoxy groups is located at δ 4.11 ppm. In the spectrum of quinone **6** the signal of the ring proton is located at δ 6.75 ppm being a singlet. The signal of the OH proton is located at δ 4.24 ppm being a triplet due to spin coupling with the OCH_2 protons ($^3J_{HH}$ = 5.7 Hz). Their resonance appears at δ 3.88 ppm as a quartet due to the nearly equal spin couplings with the OH and SCH_2 protons ($^3J_{HH}$ ~6 Hz). The signal of the SCH_2 protons is located at δ 3.13 ppm being a triplet due to spin coupling with the OCH_2 protons ($^3J_{HH}$ = 6.2 Hz).

2.2. Biological studies

At the first step of evaluation of biological properties of compounds **1–8**, we have analyzed their ability to inhibit the growth of four mammalian cell lines; tumor cell lines from human myeloma (RPMI 8226) and human mammary adenocarcinoma (MCF-7), as well as mouse fibroblasts (LMTK) and primary mouse fibroblasts (PMF).

Fig. 1A shows representative data for compounds **2**, **3**, and **8** in the case of MCF-7 cells. The results obtained for compounds **1**–**8** with all types of cells are summarized in Table 1. It can be seen that compound **1** and **2** demonstrated the best inhibition of RPMI cancer cells (0.96–1.2 μM), but suppressed the growth of MCF-7 cells at concentrations 4.8–6.0-fold higher (5.8–6.0 μM) (Table 1). Derivative **6** had IC_{50} (2.9 μM) for RPMI cells 4.4-fold lower than that for MCF-7 cells (12.8 μM). Compounds **4** and **5** inhibited the growth of cells of both cancer cell lines at nearly the same concentrations (5.1–7.5 μM) (Table 1). Derivative **7** demonstrated IC_{50} (4.0 μM) for RPMI 1.8-fold lower than compound **8** (7.1 μM), while 50% inhibition of MCF-7 cells for both compounds (IC_{50} = 26.5–47.1 μM) was observed at 6.6-fold higher concentrations than for RPMI cells. Only compound **3** suppressed the growth of MCF-7 cells (2.4 μM) at a lower concentration (5.6 μM) than that for RPMI cells. Average values of the efficiency of suppressing of two lines of tumor cells decreased from compound **1** to **8** (Table 1).

Antitumor drugs may be considered potentially more useful when they are better suppressors of tumor than normal mammalian cells. Therefore, we have compared the effects of these compounds on cancer cell lines and mouse fibroblasts LMTK, and primary mouse fibroblast cell line (PMF). Interestingly, all eight compounds suppressed the growth of normal cells at higher concentrations than was observed for tumor cells. Compound **1** inhibited the growth of LMTK (11.7 μM) and PMF (3.3 μM) cells at concentrations 2.8–9.8-fold higher than the growth of RPMI cells, while its effects on normal cells were comparable with that on MCF-7 cells (5.8 μM) (Table 1). Compound **4** inhibited the growth of LMTK cells by 50% at 30 μM, whereas the growth of PMF cells was suppressed only by 45% at significantly higher concentrations (25 μg/ml or 75.0 μM) (Table 1). The ratios of concentrations inhibiting the growth of the two types of normal and two types of tumor cells were: 0.57–9.8 (compound **1**), 1.4–18.9 (**2**), (4.4–18.5 (**3**), 5.0–12.5 (**4**), 4.5–10.3 (**5**), 2.0–10.3 (**6**), 0.34–3.4 (**7**), and 1.0–7.4 (**8**). Thus, the best difference in inhibition of tumor and normal cells was observed for compounds **3**, **4**, and **5**.

Recently we have compared the growth-inhibiting properties of F-Cpd5 with those for eleven new polyfluoro-1,4-naphthoquinone

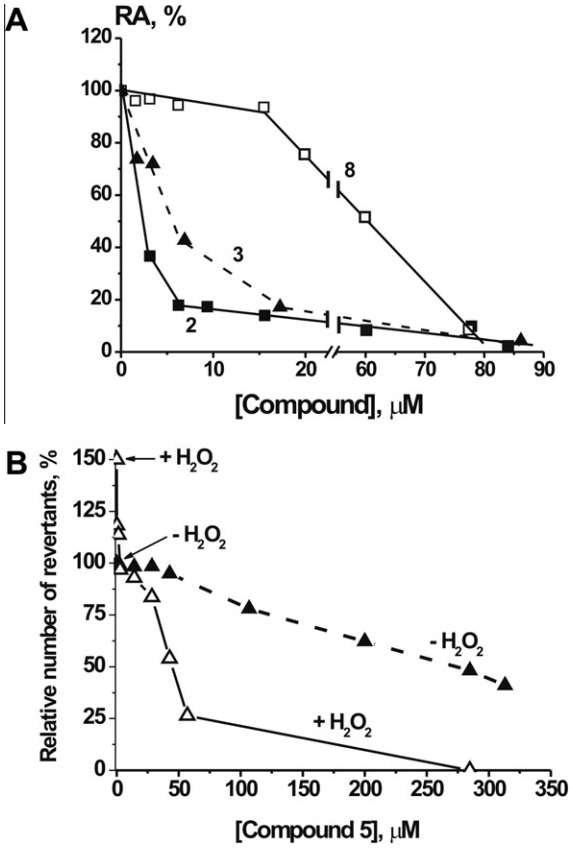


Figure 1. Effects of fluorinated compounds **2**, **3**, and **8** (relative activity, RA) on the growth of MCF-7 cells (A). Analysis of the mutagenic and antioxidant activity of compound **5** by a standard Ames test using the *S. typhimurium* strain TA102 in the absence and in the presence of 3 mM H_2O_2 (B). The number of revertants in the absence of H_2O_2 was taken for 100%. The average error in three experiments for any compound concentration did not exceed 5–15%.

derivatives.[15,16] Seven of them: 2-*n*-butylamino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (I), (2,2′-dithiodi-2)-3,5,6,7,8-pentafluoro-1,4-naphthoquinon-2-ylamino)ethane (II), 2-(2′-methylthioethyl)amino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (III),[15] 2-*tert*-butylamino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (IV), 2-diethylamino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (V), 2-ethylamino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (VI), and 2-phenylamino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (VII)[16] exhibited IC_{50} 2–72-fold lower than that for F-Cpd5 (IC_{50} = 14.8 ± 0.9 μM for RPMI and 173.0 ± 21.0 μM for MCF cells).[15,16]

The structures of **1**–**8** resemble that of F-Cpd5 and above mentioned naphtoquinones (I–VII), therefore one could expect similar effects of all these compounds on the Cdc25 phosphatases and growth of tumor cells. For RPMI cancer cells, F-Cpd5 revealed IC_{50} 2.1–14.8-fold higher than IC_{50} of compounds **1**–**8**, while all compounds **1**–**5** inhibited the growth of MCF cells 13–72-fold better than F-Cpd5 (Table 1). Notably, compound **6** differs from F-Cpd5 only by having a hydrogen atom instead of a methyl group in position 3, but derivative **6** suppressed the growth of RPMI cells 5.3-fold better than F-Cpd5. The data suggest a common mechanism of cancer cell growth inhibition by F-Cpd5[10–14] and compounds **1**–**8**.

It is known that some compounds interacting with many cell targets at the same time may be polyfunctional and possess cytoprotective properties, or, on the contrary, may be cytotoxic, mutagenic or carcinogenic. Obviously, drugs are more successful when they are not mutagenic at least at the therapeutic concentrations. The *Salmonella typhimurium* TA102 strain is often used both for evaluation of mutagenicity of different compounds and for detection of antioxidant properties, as judged from suppression of spontaneous mutagenesis in this strain and from a decrease in mutagenicity of oxidants, usually H_2O_2.[19] The mutagenic activity of compounds **1**–**8** was estimated in the Ames test[19] using *S. typhimurium* TA102 as reported by Kemeleva et al.[20] The mutation induction in the Ames assay is estimated by calculating the frequency of reversion from histidine auxotrophy to prototrophy in response to the substance under testing.[19,20]

Fig. 1B shows the representative data for quinone **5**. Compounds **1**, **3**, **6**, and **7** displayed higher potency (IC_{50} values 26–54 μM) in suppression of the spontaneous appearance of mutants than derivatives **2**, **4**, **5**, and **8** (153–275 μM) (Table 2). Some antioxidant compounds are known to efficiently decrease the mutagenic effect of H_2O_2.[19,20] In the Ames test, H_2O_2 was added to TA102 cells at the optimal concentration, 3 mM,[19,20] and the test compound concentrations varied (Fig. 1B). At low concentrations (2.8–3.4 μM) only derivatives **3**, **5**, and **7** efficiently suppressed the H_2O_2-dependent formation of mutants from 150% to 100% of revertants (the number of revertants observed in controls without

Table 1
Cytotoxicity (IC_{50}) of the fluorinated derivatives of 1,4-naphthoquinone

No.	Compound	IC_{50} (μM) for different cell lines[a]					
		RPMI	MCF-7	Average value	LMTK	PMF	Average value
1	2-(2′-Hydroxy-ethylamino)-3,5,6,7,8-pentafluoro-1,4-naphthoquinone	1.2 ± 0.3	5.8 ± 1.6	3.5 ± 1.0	11.7 ± 1.6	3.3 ± 0.8	7.5 ± 1.2
2	2,3-Dimethoxy-5,6,7,8-tetrafluoro-1,4-naphthoquinone	0.96 ± 0.05	6.0 ± 1.4	3.6 ± 0.7	8.6 ± 0.7	18.9 ± 14.8	13.8 ± 7.8
3	2-Methyl(2′-hydroxyethyl)amino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone	5.6 ± 0.3	2.4 ± 0.3	4.0 ± 0.3	44.5 ± 9.3	24.9 ± 5.3	34.7 ± 7.3
4	2-Morpholino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone	6.0 ± 0.5	5.7 ± 0.6	5.9 ± 0.6	30.0 ± 4.5	Inh. 45%[b]	≫53.0
5	2-Bis-(2′-hydroxyethyl)amino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone	5.1 ± 0.2	7.5 ± 0.2	6.2 ± 0.6	52.7 ± 11.6	34.0 ± 7.1	43.4 ± 9.3
6	5,6,7,8-Tetrafluoro-2-(2-hydroxy-ethylsulfanyl)-[1,4]naphthoquinone	2.9 ± 0.3	12.8 ± 3.0	7.9 ± 1.7	25.0 ± 5.0	30.0 ± 10.0	27.5 ± 8.0
7	3,5,6,7,8-Pentafluoro-2-methoxy-1,4-naphthoquinone	4.0 ± 0.6	26.5 ± 5.4	15.3 ± 3.0	13.7 ± 1.0	8.9 ± 2.9	11.3 ± 1.5
8	*N*-(1,4-Dioxo-3-hydroxy-2-tetrafluoro-1,4-dihydronaphthyl) pyridinium betaine	7.1 ± 0.5	47.1 ± 5.8	27.1 ± 3.1	46.4 ± 2.8	52.6 ± 13.9	49.5 ± 8.4
9	Control compound F-Cpd5[16,17]	14.8 ± 0.9	173.0 ± 21.0	93.9 ± 11	No inhibit at 173 μM	nd[c]	nd

[a] Mean ± SD from three independent experiments.
[b] When the cytotoxicity was low, the percent of inhibition of cell growth at the highest used concentration (25 μg/ml) of the compound was determined.
[c] nd, not determined.

Table 2
IC_{50} values characterizing suppression of spontaneous and H_2O_2-induced mutagenesis by polyfluorinated derivatives of 1,4-naphthoquinone

No.	Compound	IC_{50}[a] (μM)		
		Suppression of spontaneous mutagenesis (from 100% to 50%)	Suppression of H_2O_2-induced and spontaneous mutagenesis	
			From 150% to 100%	From 100% to 50 %
1	2-(2′-Hydroxy-ethylamino)-3,5,6,7,8-pentafluoro-1,4-naphthoquinone	54.0 ± 5 .4	26.0 ± 2.6	44.9 ± 4.5
2	2,3-Dimethoxy-5,6,7,8-tetrafluoro-1,4-naphthoquinone	198.1 ± 18.6	38.3 ± 3.5	53.8 ± 5.5
3	2-Methyl(2′-hydroxyethyl)amino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone	37.6 ± 3.8	3.4 ± 0.3	30.2 ± 3.0
4	2-Morpholino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone	131.4 ± 12.9	54.0 ± 6.0	168.9 ± 16.0
5	2-Bis-(2′-Hydroxyethyl)amino-3,5,6,7,8-pentafluoro-1,4-naphthoquinone	275.3 ± 27.5	2.9 ± 0.3	45.3 ± 4.5
6	5,6,7,8-Tetrafluoro-2-(2-hydroxy-ethylsulfanyl)-[1,4]naphthoquinone	26.1 ± 2.6	12.7 ± 1.3	23.5 ± 2.6
7	3,5,6,7,8-Pentafluoro-2-methoxy-1,4-naphthoquinone	39.0 ± 3.9	2.8 ± 0.3	20.0 ± 2.1
8	*N*-(1,4-Dioxo-3-hydroxy-2-tetrafluoro-1,4-dihydronaphthyl) pyridinium betaine	153.0 ± 15.3	61.8 ± 6.2	185.4 ± 18.5
9	Control compound F-Cpd5: 5,6,7,8-tetrafluoro-2-(2-mercaptoethanol)-3-methyl-[1,4]naphtoquinone[16,17]	0.81 ± 0.1	nd[b]	0.44 ± 0.07

[a] Mean ± SD from three independent experiments.
[b] nd, not determined.

H_2O_2 was taken for 100%), while derivatives **1**, **2**, **4**, **6**, and **8** did it at significantly higher concentrations (12.7–61 μM) (Table 2). The ratio of the concentrations corresponding to a decrease in mutant appearance from 150% to 100% and from 100% to 50% in the presence of H_2O_2 depended significantly on the compound: 1.7 (compound **1**), 1.4 (**2**), 8.9 (**3**), 3.1 (**4**), 5.6 (**5**), 1.9 (**6**), 7.1 (**7**), and 3.0 (**8**). These data indicate that compounds **1**–**8** are not mutagenic themselves and decrease the level of spontaneous mutagenesis as well as the mutagenic effect of H_2O_2.

Interestingly, in the absence of H_2O_2 the potency of compounds **5** and **2** in suppressing spontaneous (IC_{50} = 198–275 μM) was 3.7–6.0-fold lower than in decreasing H_2O_2-dependent mutagenesis (IC_{50} = 45.3–53.8 μM), while other compounds showed in some extent comparable IC_{50} values in the presence and in the absence of H_2O_2 (Table 2). Since compounds **1**–**8** did not enhance the spontaneous mutagenesis and effectively suppressed the mutagenic effect of H_2O_2, they can be considered efficient antioxidants. At the same time, it was not possible to exclude that the complete suppression of mutant cell appearance by compounds **1**–**8** at high concentration may result not only from their ability to suppress spontaneous and H_2O_2-induced mutagenesis but also from their higher general toxicity towards bacterial cells. However, the 50% decrease in spontaneous and H_2O_2-dependent mutagenesis of bacterial cells including their possible general toxicity for all compounds was observed at significantly higher concentrations than those having an effect in tumor cells. The ratio of IC_{50} values in bacterial and cancer cells was 8.7–45 (compound **1**), 23.9–206 (**2**), 6.1–6.7 (**3**), 21.9 (**4**), 44–54 (**5**), 2.2–9.0 (**6**), 1.6–9.8 (**7**), and 6.6–21.5 (**8**) (Tables 1 and 2).

While the control F-Cpd5 possessed relatively low activity toward tumor cells (IC_{50} = 14.8 μM), in contrast to compounds **1**–**8** it suppressed spontaneous (IC_{50} = 0.81 μM) and especially H_2O_2-indiced mutagenesis (IC_{50} = 0.44 μM) at concentrations 18.3–33.6-fold lower. One cannot exclude that F-Cpd5 may be metabolized or may react with components of mammalian cells or cell peroxides to yield products with a higher protective function or general cytotoxicity. At the same time, in the case of polyfluorinated compounds **1**–**8** and especially **1, 2**, **4**, and **5**, the suppression of tumor cells growth may be achieved at concentrations significantly lower than those showing antioxidant and possible general toxic effects.

Our data suggest that compounds **1**–**8** demonstrate a similar or more pronounced decrease in tumor cells growth in comparison with control F-Cpd5, a potent inhibitor of Cdc25 phosphatases.[12–14] It is possible that all these compounds including F-Cpd5 play a double role, acting both as inhibitors of cell phosphatases and as antioxidants. In addition, our data suggest that all eight new compounds can be less cytotoxic than F-Cpd5 toward mammalian and bacterial cells, without being associated with the inhibition of Cdc25 phosphatases.

Fluorinated derivatives of 1,4-naphthoquinone are less active in generating ROS and may be promising inhibitors of Cdc25 as compared to 1,4-naphthoquinone.[12–14]

2.3. Conclusion

Our data indicate that compounds **3**, **4**, **5**, and **6** are the most promising, demonstrating better cytotoxic effect against cancer cells as compared with normal mammalian cells. Quinones **1**, **2**, **4**, and **5** are antioxidants protecting cells from spontaneous mutagenesis and they can possess minimal general toxicity towards different cells. In addition, **3**, **5**, **6**, and **7** are the best protectors of bacterial cells from H_2O_2-dependent mutagenesis. Taken together, our data suggest that compounds **3**–**5** may be considered as potentially useful inhibitors of growth of tumor cells, at the same time not excluding that derivatives **1**, **2**, **6**, **7**, and **8** can also be used as perspective suppressors of tumor cells.

3. Experimental

3.1. Chemistry

Commercially supplied 2-mercaptoethanol, methanol and Et_3N were purified by distillation. Quinones **7** and **9** were prepared accordingly to reported methods.[17,18] ^{19}F and ^{1}H spectra were recorded on a Bruker AV-300 NMR spectrometer at 282 MHz (^{19}F) or 300.13 MHz (^{1}H) and calibrated according to the chemical shifts of hexafluorobenzene and chloroform-*d* or acetone-d_6 for quinones **2** and **6**, respectively.

3.1.1. Procedures for the synthesis of quinones 2 and 6

3.1.1.1. 2,3-Dimethoxy-5,6,7,8-tetrafluoro-1,4-naphthoquinone 2. A mixture of quinone **7** 0.20 g (0.75 mmol), Et_3N 0.165 g (1.63 mmol) and methanol (3 ml) was stirred for 48 h at room temperature. The solvent was distilled off in vacuo. The product was purified by crystallization from methanol to yield yellow crystals (0.142 g, 65%), mp 146.5–147.5 °C. ^{19}F NMR: δ/ppm 23.9 (m, F^5, F^8), 17.3 (m, F^6, F^7); ^{1}H NMR: δ/ppm 4.11 (s, 6H, $2CH_3$). Elemental Anal. Calcd for $C_{12}H_6F_4O_4$: C, 49.67; H, 2.08; F, 26.19. Found: C, 50.03; H, 2.10; F, 26.50.

3.1.1.2. 2-[(2-Hydroxy)ethylsulfanyl)]-5,6,7,8-tetrafluoro-1,4-naphthoquinone 6. A mixture of quinone **9** 0.103 g (0.40 mmol), 2-mercaptoethanol 0.075 g (0.97 mmol) and methanol (~5 ml) was stirred for 30 min at room temperature. After purification as described above for quinone **7**, quinone **6** was obtained as yellow crystals (0.098 g, 80%), mp 171–175 °C. ^{19}F NMR: δ/ppm 23.8 (dt, F^8, $J_{8,5}$, $J_{8,6}$ ~12 Hz, $J_{8,7}$ = 19.0 Hz), 22.4 (ddd, F^5, $J_{5,6}$ ~19 Hz, $J_{5,7}$ ~10 Hz, $J_{5,8}$ – 12.5 Hz), 16.7 (dt, F^6, $J_{6,5}$, $J_{6,7}$ ~19 Hz, $J_{6,8}$ = 11.2 Hz), 14.6 (dt, F^7, $J_{7,6}$, $J_{7,8}$ ~19 Hz, $J_{7,5}$ = 9.9 Hz); ^{1}H NMR: δ/ppm 6.75 (s, 1H, CH), 4.24 (t, 1H, $^3J_{HH}$ = 5.7 Hz, OH), 3.88 (q, 2H, $^3J_{HH}$ ~6 Hz, CH_2), 3.13 (t, 2H, $^3J_{HH}$ = 6.2 Hz, CH_2). Elemental Anal. Calcd for $C_{12}H_6F_4O_3S$: C, 47.06; H, 1.97; F, 24.82; S, 10.47. Found: C, 47.13; H, 2.05; F, 24.81; S, 10.70.

3.2. Biological experiments

3.2.1. Determination of mutagenisity of compounds

In the Ames test, the histidine-dependent strain of *S. typhimurium* TA102 was used, which carries a mutation at the histidine operon.[19] The mutagenic activity of the samples was analyzed by the standard method without metabolic activation.[19] A liquid culture of TA102 was obtained by 16-h growth of cells from a frozen stock at 37 °C in LB medium with penicillin. Then cells were plated on minimal glucose agar, antibiotics and histidine at the density sufficient to obtain isolated colonies. A separate bacterial colony was inoculated into LB medium (5 ml) containing ampicillin (50 μg/ml) and tetracycline (2 μg/ml), and grown with shaking (130 rpm) for 15 h at 37 °C.

The Ames test was carried out using the double-layer method as described in.[19,20] The overnight culture of bacteria (100 μl) containing one of the tested compounds in different concentrations and, if required, 3 mM H_2O_2, were mixed at 42 °C with 2 ml of liquid 0.6% top agar. The mixture was poured onto plates with a minimal medium containing 0.2% glucose and 3% agar, taking care to distribute the mixture uniformly on the surface of the solid agar. The plates were incubated for 48 h at 37 °C, and the revertants were counted. The cells incubated with H_2O_2 in the absence of compounds analyzed were used as positive controls, and the cells grown in the absence of H_2O_2 and antioxidants served as negative controls for mutation induction. The results are expressed as mean ± standard deviation of at least three independent experiments.

3.2.2. Cytotoxicity assays

Tumor cell lines from human myeloma RPMI 8226, human mammary adenocarcinoma MCF-7, mouse fibroblasts LMTK and primary mouse fibroblast cell line (PMF) (~2000 cells per well) were incubated for 24 h at 37 °C in IMDM or RPMI 1640 medium (5% CO_2) and then were treated with compounds **1**–**8**. After 72 h of cell incubation, the relative amount of live cells was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (a standard colorimetric MTT-test[21]) and the drug concentration that caused 50% cell growth inhibition (IC_{50}) was determined. The results are expressed as mean ± standard deviation of at least three independent experiments.

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