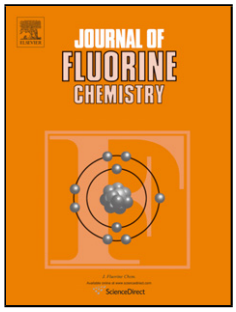

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Phosphonium Betaines Derived from Hexafluoro-1,4-naphthoquinone: Synthesis and Cytotoxic and Antioxidant Activities

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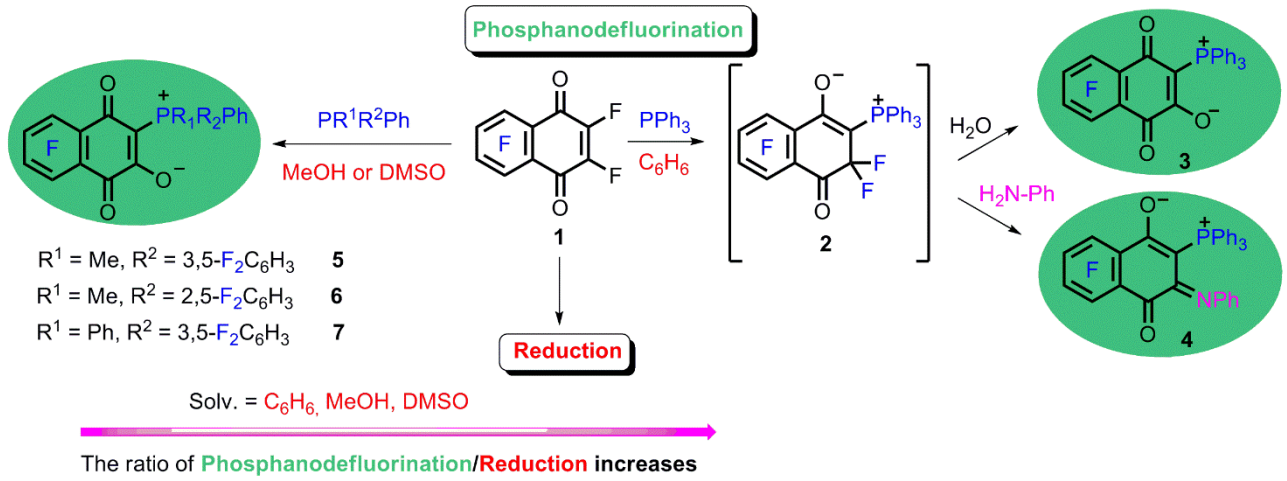
Graphical abstract



Abstract: Fluorinated derivatives of 1,4-naphthoquinones are highly potent inhibitors of Cdc25A and Cdc25B phosphatases; they suppress the growth of tumor cells. Four derivatives of phosphonium betaines derived from hexafluoro-1,4-naphthoquinone: (triphenyl[5,6,7,8-tetrafluoro-1-oxido-4-oxo-3-(phenylimino)-3,4-dihydronaphthalen-2-yl]phosphonium) (**4**), ((3,5-difluorophenyl)(methyl)phenyl(5,6,7,8-tetrafluoro-3-oxido-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phosphonium) (**5**), ((2,5-difluorophenyl)(methyl)phenyl(5,6,7,8-tetrafluoro-3-oxido-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phosphonium) (**6**) and ((3,5-difluorophenyl)diphenyl(5,6,7,8-tetrafluoro-3-oxido-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phosphonium) (**7**) were synthesized for the first time. Their cytotoxicity toward human mammary adenocarcinoma, human myeloma, hamster and murine and fibroblasts as well as their antioxidant and mutagenic effects on a *Salmonella* tester strain were analyzed. All four substances showed comparable IC_{50} values in terms of suppression of tumor cell growth, which were from two- to ninefold lower comparing with those of fibroblasts. To identify the features of spatial orientation and noncovalent interactions of the new phosphonium betaines in the binding site of Cdc25B, a molecular docking analysis was carried out. It showed that the interactions of the analyzed compounds with a Cdc25B model binding site are characterized by the presence of a large number of acceptors (fluorine and oxygen atoms, forming halogen and hydrogen bonds) and by participation of pi-systems and phosphorus in specific electrostatic interactions that may result in inhibition of enzymes of the Cdc25 family. In addition, compounds **5** and **6** (especially the latter) were found to be effective antioxidants protecting bacterial cells from H_2O_2-induced and spontaneous and mutagenesis at significantly lower concentrations (IC_{50} = 0.09 to 1.8 μM) than those of derivatives **4** and **7** (86–92 μM). Taking into account these data (together with the good cytotoxic effect on cancer cells comparing with normal mammalian cells) we can propose compounds **5** and **6** as possible useful inhibitors of tumor cell growth and antioxidants.

Keywords: Fluorinated 1,4-naphthoquinones, triphenylphosphanes, phosphonium betaines, cytotoxicity.

1. Introduction

Mammalian cell-to-cell communication and division are regulated by phosphorylation of proteins, which is the result of a dynamic equilibrium between the activity of protein kinases and phosphatases. The subfamily of dual-specificity phosphatases of proteins retains some of the structural attributes of post-translational modifications of proteins, but these enzymes have a unique ability to hydrolyze both phospho-serine/-threonine and phosphotyrosine residues on the same protein substrate. Key members of this family of dual-specificity phosphatases are Cdc25 phosphatases controlling the cell cycle. Phosphatases of the Cdc25 subfamily remove phosphate groups from cyclin-dependent kinases (CDKs) and thereby activate complexes cyclin-CDK regulating the cell cycle [1,2]. Cdc25A and Cdc25B are strongly expressed in many tumor cells, frequently showing correlations with a higher grade or aggressiveness of tumors and unfavorable prognosis [3]. The hypothetical involvement of Cdc25 phosphatases in tumor cells growth makes these cell cycle-regulatory proteins possible therapeutic targets in cancer [4,5].

Small-molecule inhibitors have served as effective tools for elucidation of the function of kinases and phosphatases involved in certain cellular signaling pathways because the effects of such inhibitors are usually nonquantal and reversible, and these molecules easily penetrate the plasma membrane. Some of the many tested substances have been shown to suppress activity of the enzyme of Cdc25 subfamily [6,7], in particular, NSC 95397 (2,3-bis[(2-hydroxyethyl)thio]-1,4-naphthoquinone (National Cancer Institute Library) was demonstrated to be the strongest inhibitor of Cdc25 [8]. 7-Aminoquinoline-5,8-quinones and *p*-naphthoquinones represent the backbones for the synthesis of possible Cdc25 inhibitors [8, 9], including NSC 663284 [10]. For instance, Cpd5, or 2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone, was found to be an effective inhibitor of Cdc25 phosphatases [11].

Most quinones have been shown to suppress Cdc25 activity via sulfhydryl arylation in the quinone moiety because of redox properties, but they can also cause formation of toxic species of oxygen [12], which may be harmful to healthy tissues and thereby reduce the therapeutic utility of quinones. One possible way to overcome the inherent cytotoxicity of quinones is to use derivative of compounds that are stable when reduced and therefore pose a lower risk of the formation of radicals and resultant nonspecific damage to cells. Fluorinated derivatives of 1,4-naphthoquinones are less likely to cause formation of reactive oxygen species (ROS); among several candidates, fluorinated Cpd5 – 5,6,7,8-tetrafluoro-2-(2-mercaptoethanol)-3-methyl-[1,4]-naphthoquinone (F-Cpd5) was found to be the strongest inhibitor of Cdc25A and Cdc25B phosphatases [13-14,15].

Calculations predicted F-Cpd5 to have much more potent reductive properties comparing with Cpd5 without generation of ROS [13]. F-Cpd5 is threefold more effective than Cpd5 in suppressing the growth of human hepatoma Hep3B cells [13-15] and inhibits mitogen-stimulated DNA replication 12-fold less strongly in healthy rat hepatocytes than in Hep3B cells [14].

The rationale aim in the research of Cdc25 phosphatases inhibitors is the search for better inhibitors of tumor growth that have no mutagenic, carcinogenic, and cytotoxic effects. Because all naphthoquinones are inhibitors of Cdc25 phosphatases [8-15], it is only logical to hypothesize that some new polyfluorinated naphthoquinones should also inhibit these phosphatases. Accordingly, we recently synthesized several novel polyfluorinated phenylamino-, *n*-alkylamino-, and alkylthioderivatives of 1,4-naphthoquinone and characterized their antioxidant and mutagenic properties by means of a special type of *Salmonella* cells as well as their toxicity toward primary mouse fibroblasts comparing with murine and human tumor cells [16=17,18]. It is noteworthy that for all these compounds, the effectiveness of inhibition of cancer cell growth at significantly lower concentrations in comparison with healthy cells as well as the mutagenic and antioxidant activities in the above-mentioned bacterial system substantially depend on the structure of functional groups; only three or four of these compounds are promising, i.e., have all of these important properties [16-18].

In the present study, we carried out experiments to synthesize four novel phosphonium betaine derivatives of polyfluorinated 1,4-naphthoquinones including one with imino-functionality and several compounds with the fluorinated phenyl-substituent attached to a phosphorous atom. We compared these compounds in terms of specific toxicity toward cancer cells and prevention of spontaneous and H_2O_2-induced mutagenesis in bacterial cells.

2. Results and Discussion

2.1. Reactions of hexafluoro-1,4-naphthoquinone with PPh_3 and fluorophenylphosphanes

In our first report [19] we proposed that the reaction of hexafluoro-1,4-naphthoquinone (**1**) with PPh_3 in MeOH initially yields phosphonium salt **A**, in which a triphenylphosphonium group effectively activates the neighboring position 3 for a nucleophilic attack, whereupon the rapid F^3 substitution by methanol takes place to produce (after demethylation) phosphonium betaine **3** with a ~90% yield. To prove or to refute this idea, we conducted a reaction of hexafluoro-1,4-naphthoquinone **1** with PPh_3 (1:1) in C_6H_6 instead of MeOH (Scheme 1). In this case, the reaction also produced phosphonium betaine **3** with a slightly lower (75%) yield comparing with the reaction in MeOH. The second product that we isolated was also Ph_3PO (~25%); its formation indicated that the triphenylphosphanodefluorination reaction competed with reduction of the quinone moiety.

Because under normal conditions, the products of reaction of quinone **1** with PPh_3 in C_6H_6 and in MeOH were identical, for detection of the reaction intermediates, we designed a series of specific experiments, which were carried out in thoroughly dried benzene in an atmosphere of dry argon. The results were obtained rapidly (Scheme 1); in the ^{19}F and $^{31}P\{^1H\}$ NMR spectra of the solution immediately after mixing of quinone **1** with PPh_3, we detected signals not only of betaine **3** but also (3,3,5,6,7,8-hexafluoro-1-oxido-4-oxo-3,4-dihydronaphthalen-2-yl)triphenylphosphonium (**2**) and Ph_3PF_2 (38%, 54%, and 8%, respectively). In the NMR ^{19}F spectrum, the unusual in its structure betaine **2** showed five multiples in the intensity ratio 2:1:1:1:1 respectively at δ_F –77.6 ppm (br. s, 2F, CF_2), –138.8 and –139.2 ppm (ddd, 1F, $^3J_{FF} \approx 20$, $^4J_{FF} \approx 10$, and $^5J_{FF} \approx 13$ Hz; $F^{5,8}$), –144.9, and –151.0 ppm (ddd, 1F, $^3J_{FF} \approx 20$, $^4J_{FF} \approx 10$ Hz; $F^{6,7}$). In the NMR $^{31}P\{^1H\}$ spectrum of the solution, the singlet at δ_P 16.8 ppm belonged to compound **2**. As for the known PPh_3F_2 [20], its signals were readily identified in the reaction mixture: a doublet at δ_F –39.1 ppm with $J_{FP} \approx 665$ Hz and a triplet at δ_P –54.5 ppm $J_{PF} \approx 665$ Hz were observed in the NMR ^{19}F and $^{31}P\{^1H\}$ spectra correspondingly.

Thus, these experiments revealed two important products in the reaction of **1** with PPh_3: betaine **2** and PPh_3F_2. In structure **2**, the $>CF_2$ fragment is present, from which we can hardly expect high reactivity. Therefore, it can be assumed that betaine **2** is in rapid equilibrium with minor intermediate **A**, which, in turn, easily undergoes hydrolysis even in the presence of traces of water. The formation of PPh_3F_2 indicates a parallel reaction of reduction of quinone **1** by PPh_3 [21]. In general, in all these experiments, the process of phosphanodefluorination mostly dominated over reduction [ratio (**2**+**3**)/$PPh_3F_2 \approx 12:1$], that did not allow us to register the corresponding reduction intermediate of type **B**. The formation of last was detected in case of chloranil where the reduction process dominates [22].

To confirm the intended **A⇆2** equilibrium, the reaction of quinone **1** with PPh_3 in dried C_6H_6 was carried out followed by treatment with aniline (Scheme 1). According to ^{19}F and $^{31}P\{^1H\}$ NMR data, along with omnipresent betaine **3** (~30%),[†] the reaction also resulted in the formation of previously unknown triphenyl[5,6,7,8-tetrafluoro-1-oxido-4-oxo-3-(phenylimino)-3,4-dihydronaphthalen-2-yl]phosphonium (**4**, ~50%, isolated yield 36%), whose structure was unambiguously solved by means of NMR and XRD data. The X-ray molecular structure and selected bond lengths are depicted in Figure 1. The bond lengths and bond angles are the same as the statistical means [23]. In solid **4**, the benzoquinone ring has an envelopelike conformation

[†] The formation of betaine **3** is result of hydrolysis of intermediate **A** by atmospheric moisture during working-up procedure.

characterized by a 0.289(4) Å deviation of the C3 atom from the plane of the other atoms (with standard deviations 0.030 Å). In the crystal, the C–F...π, C=O...π, and C–H...π interactions lead to formation of 3D networks. The atom-to-plane distances are 3.23 Å and 3.18 Å for interactions between atoms F1, O2, and the fluorinated ring, and 2.89 Å for interactions between atom H5 and phenyl ring C11–C16 (Fig. 2).

Previously, it was found that betaine **3** and its derivatives can effectively inhibit growth of cancer cell (for RPMI 6228 cells, IC_{50} reached 3.4 ± 0.3 μg/ml) [24]. At the same time, they show substantial antioxidant activities, which make this type of compounds good candidates for use in anticancer treatments [19]. In the present work, we tried to extend the range of type **3** compounds by means of fluorinated phosphane PR^1R^2Ph (Scheme 2). Experiments revealed that in reactions of **1** with PR^1R^2Ph, the corresponding betaines formed successfully but with a low yield owing to the greater predisposition of the analyzed phosphanes to oxidation. Accordingly, the reaction of quinone **1** with (3,5-difluorophenyl)(methyl)phenylphosphane $PMe(3,5\text{-}F_2C_6H_3)Ph$, along with the desired betaine (3,5-difluorophenyl)(methyl)phenyl(5,6,7,8-tetrafluoro-3-oxido-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phosphonium (**5**) (~60%, isolated yield 38%), produced a substantial amount of the corresponding phosphane oxide (~40%). Fortunately, it was found that the use of DMSO as a solvent noticeably increased the phosphanodefluorination reaction rate and, consequently, raised the yields of betaines. For example, the reaction of quinone **1** with (2,5-difluorophenyl)(methyl)phenylphosphane or (3,5-difluorophenyl)diphenylphosphane resulted in reaction mixtures containing betaines (2,5-difluorophenyl)(methyl)phenyl(5,6,7,8-tetrafluoro-3-oxido-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phosphonium (**6**) or (3,5-difluorophenyl)diphenyl(5,6,7,8-tetrafluoro-3-oxido-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phosphonium (**7**) (with 90% NMR yields), from which they were isolated with the yield of 67% and 57%, respectively (Scheme 2).

2.2. Biological experiments

Naphthoquinone betaines **4–7** were analyzed for their ability to inhibit the growth of three mammalian cell lines: human myeloma (RPMI 8226), tumor cell lines from human mammary adenocarcinoma (MCF-7), and a hepatocellular carcinoma epithelial tumor (HepG2), as well as normal Chinese hamster Ag 17 cells (AG) and healthy murine fibroblasts (LMTK).

At the first step of biological assessment of compounds **4–7**, we study their ability to suppress the growth of three tumor cell lines rather than mouse fibroblasts. Figure 3 shows representative

results on these compounds in the case of HEP, RPMI, and AG cells. The data obtained for compounds **4–7** with all types of cells are shown in Table 1.

One can see that compounds **4–7** showed more or less comparable IC_{50} values in the case of MCF-7 (4.7–8.5 μM), RPMI (3.6–5.5 μM), and HEP (4.5–10.6 μM) cancer cells. Antitumor drugs are regarded as potentially more effective when they are stronger growth suppressors of tumor cells comparing with healthy mammalian cells. Consequently, we compared the actions of these compounds on cancer cell lines and normal mouse (LMTK) and hamster (Ag-17) fibroblasts. All four compounds inhibited the growth of healthy cells at 1.0- to 9.1-fold higher concentrations relative to tumor cells. The biggest difference between the inhibition of growth of tumorous and healthy cells was observed for compounds **5** and **6** (Table 1).

Recently, we compared the growth-inhibitory effects of F-Cpd5 with those of 19 novel containing no phosphorus atoms polyfluoro-1,4-naphthoquinone derivatives [16-18]. Fifteen of them suppressed the growth of tumor cells at a 2- to 72-fold lower concentration in comparison with F-Cpd5 (IC_{50} = 14.8 ± 0.9 μM for RPMI cells and 173.0 ± 21.0 μM for MCF cells) [16-18]. IC_{50} values of compounds **4–7** are also ~1.9- to 2.7-fold and 30- to 38-fold less than those of F-Cpd5 in the case of RPMI and MCF cells, respectively (Table 1).

To clarify the details of spatial orientation and noncovalent interactions of the novel phosphonium betaines derived from hexafluoro-1,4-naphthoquinone in the binding site of Cdc25B, a molecular docking analysis was conducted. Previously, several studies have revealed two catalytic binding sites in Cdc25B structure that are available for interaction with small molecules [25,26]. The first one (His472 through Arg479) has a relatively compact cavity and can bind only small anions. The second binding site is composed of residues 379–380, 397–399, 471, 484–485, 488–489, 492, 500, 503, 505, and 507 and has a binding cavity suitable for polycyclic molecules. The conserved structure of this region in the group of dual-specificity phosphatases, the anion binding, and the reactive sulfhydryl group of Cys484 indicate that this region performs some important function [25]. Predictions of binding modes of the phosphonium betaines under study are presented in Figure 4.

The predicted spatial orientation of molecules analyzed in the binding site depends on structure of the substituents. Compounds **4**, **5**, and **6** have the orientation of the naphthoquinone core toward the outer hydrophilic area of the binding site, but compound **4** has an inverted conformation of the naphthoquinone core. We observed a difference in orientations of the substituents in the inner hydrophobic region of the binding site. For compound **4**, this region is occupied by the

iminophenylic group of the naphthoquinone core, and the triphenylphosphonium part of the molecule is not involved in the interactions. As for compounds **5** and **6**, the inner region is occupied by one of substituents of phosphonium: phenyl (**5**) and 2,5-difluorophenyl (**6**). Compound **7** is inserted into the inner portion of the binding site by the naphthoquinone core. Fluorine atoms forming halogen and hydrogen bonds with amino acid residues (Glu377, Arg485, Arg488, Arg492, Asp397, Tyr382, Cys484, and others) of the binding site are important for binding of this type of compounds to the Cdc25B model. Compounds **5** and **6** show participation of naphthoquinone in the formation of hydrogen bonds and electrostatic interactions of the positively charged phosphorus atom. It should be mentioned that π-systems of the analyzed compounds participate in electrostatic interactions with Glu377, Arg488, and Arg492. Therefore, the characteristics of the predicted interactions of the analyzed compounds with the binding site of the Cdc25B model are determined by the presence of a large number of acceptors: fluorine and oxygen atoms, forming halogen and hydrogen bonds and by participation of π-systems and phosphorus in electrostatic interactions. The reactive sulfhydryl group of Cys484 (involved in interactions with **4**, **6** and **7**) may lead to blockage of disulfide bond formation, which may be responsible for binding to another domain of the full-length Cdc25 proteins, to cyclin/kinases, or other proteins [25].

It is known that some compounds interacting with many cell targets at the same time can be polyfunctional and have cytoprotective properties, or conversely, may have mutagenic or carcinogenic effects. Obviously, drugs are more successful when they are not mutagenic at therapeutic concentrations. The mutagenic activity of compounds **4–7** was estimated by the Ames test [27] based on *S. typhimurium* TA102 as described by Kemeleva *et al.* [28]. The mutagenicity in the Ames assay is assessed by computing the frequency of reversion from histidine auxotrophy to prototrophy under the influence of the substance being tested [27,28]. Figure 5 shows the representative data on quinones **4–7**, and all results are summarized in Table 2. One can see that although all four compounds have comparable IC_{50} values for the inhibition of cancer cell growth, they have different effectiveness at suppressing the spontaneous and H_2O_2-induced mutagenesis. Compound **6** showed the highest potency (IC_{50} = 0.26 μM) of suppression of the spontaneous formation of mutants, whereas this activity for compound **5** (1.8 μM) was ~6.9-fold lower. Compounds **4** and **7** inhibited the spontaneous mutagenesis very weakly. At a relatively high concentration (50 μg/ml), compound **7** suppressed spontaneous mutagenesis only by 15.5% ± 1.2% (50 μg/ml = 92.20 μM), while compound **4** by 44.4% ± 3.5% (86 μM; Table 2).

At low concentrations (0.04–0.09 μM), all four quinones effectively decreased the H_2O_2-dependent formation of mutants: from 137.7% to 100% of revertants (the number of revertants observed in controls without H_2O_2 was set to 100%; Table 2). At the same time, the total inhibition of peroxide-induced and spontaneous mutagenesis by these compounds was quite different.

Compound **6** (IC_{50} = 0.09 μM) showed the best protection of the cells from peroxide-induced and spontaneous mutagenesis (a decrease in formation of mutants from 100% to 50% in the presence of H_2O_2), while compounds **5** (0.62 μM) and **4** (86.0 μM) were 6.9- and 956-fold less efficacious, respectively. Compound **7** was found to offer the worst protection of cells either from spontaneous or from total mutagenesis; in the presence of H_2O_2 at high concentration (50 μg/ml = 92.2 μM), it decreased formation of mutants from 100% to 50% only for 40.0% ± 3.0%. The efficiency of protection of cells from the harmful effects of H_2O_2 can ranked as follows: **6** > **5** > **4** > **7** (descending order; Table 2).

For many previously analyzed polyfluorinated alkylthio-, phenylamino-, and *n*-alkylaminoderivatives of 1,4-naphthoquinone [16-18], most often (with infrequent exceptions), the suppression of mutant formation in the presence of H_2O_2 was observed at similar or even greater concentrations than those for the suppression of spontaneous mutagenesis. Figure 5 shows that compounds **4–7** suppress the formation of mutants in the presence of H_2O_2 at concentrations lower than those in the absence of this peroxide. Moreover, all four compounds highly effectively decrease H_2O_2-driven mutagenesis (from 137.7% to 100%) at low and similar concentrations (0.04–0.09 μM) (Table 2). This finding suggests that all four compounds can effectively interact with H_2O_2 thus efficiently reducing its concentration. The bigger decrease in mutagenesis in the presence of H_2O_2 may be explained as follows: the products of H_2O_2-mediated oxidation of these compounds are more efficacious traps of oxidative compounds. Nevertheless, the four initial compounds and products of their H_2O_2-mediated modification differ substantially in the inhibition of spontaneous mutagenesis. The differences observed here can be related to the structural features of these compounds. The best antioxidants (**6** and **5**) contain the PPhMe moiety, which is absent in weaker antioxidants **4** and **7**.

These findings imply that quinones **4–7** are not mutagenic by themselves and inhibit spontaneous mutagenesis as well as mutagenic effect of H_2O_2. Because compounds **6** and especially **5** did not enhance the spontaneous mutagenesis and effectively suppressed the mutagenic effect of H_2O_2 at low concentrations, they can be considered efficient antioxidants. Our data indicate that compounds **4–7** cause a more pronounced decrease in tumor cell growth comparing with the control compound (F-Cpd5, a potent inhibitor of Cdc25 phosphatases) [13-15]. The structures of **4–7** resemble the structure of F-Cpd5 and of the above-mentioned previously described derivatives of quinones [16-18]; accordingly, we may expect similar effects of these compounds on Cdc25 phosphatases and on tumor cells growth. It is obvious that all these compounds (including F-Cpd5) perform a twofold function, acting both as inhibitors of cellular phosphatases and as antioxidants. Compounds **5** and **6** (especially the latter) suppress spontaneous and H_2O_2-mediated mutagenesis (IC_{50}) on average at concentrations 5- and 41-fold lower, respectively, than concentrations at which

they inhibit the growth of tumor cells. Taking into account the efficacy of suppression of tumor cell proliferation and antioxidant effects, compounds **6** and **5** (especially the latter) appear to be more promising inhibitors of Cdc25 than 1,4-naphthoquinone.

3. Conclusions

We studied the reaction of hexafluoro-1,4-naphthoquinone with PPh_3 in dry benzene and identified two main fluorinated intermediates: the betaine with two germinal fluorine atoms and Ph_3PF_2. Formation of these intermediates proves the existence of two parallel processes of interaction of hexafluoro-1,4-naphthoquinone with PPh_3. The first process is the phosphanodefluorination yielding the intermediate betaine, and the second one is a redox transformation. The intermediate betaine is highly reactive and in the presence of water or aniline easily yields the corresponding stable phosphonium betaines. We showed that the fluorinated phosphanes PR_1R_2Ph (R_1 = Me, Ph; R_2 = Ph, 3,5-, 2,5-$F_2C_6H_3$) also effectively participate in the reaction with hexafluoro-1,4-naphthoquinone and thus offer a variety of unknown polyfluorinated betaines.

Fluorinated derivatives of 1,4-naphthoquinone are less active in terms of production of ROS and may be promising inhibitors of Cdc25 as compared to 1,4-naphthoquinone. Our results suggest that compounds **5** and **6** have the best potential: they show stronger cytotoxic effects on cancer cells as compared to healthy mammalian cells and have the best antioxidant action on bacterial cells in the presence and absence of H_2O_2.

4. Experimental section

4.1. Materials and methods

Benzene was dried by distillation over P_2O_5 and finally stored over molecular sieves. PPh_3 was crystallized from Et_2O. Aniline was dried over CaH_2 and redistilled at 0.09 kPa. Reactions were conducted in Schlenk tubes.

The NMR spectra were recorded with a Bruker AV-300 (^{1}H: 300.13 MHz, ^{19}F: 282.36 MHz, $^{31}P\{^1H\}$: 121.49 MHz) and AV-400 (^{1}H: 400.13 MHz, $^{13}C\{^1H\}$: 100.61 MHz) spectrometers in the respective deuterated solvents relative to the residual proton chemical shifts of chloroform (δ_H 7.25 ppm, δ_C 77.00 ppm) in ^{1}H NMR spectra, Me_4Si in $^{13}C\{^1H\}$ NMR spectra, external C_6F_6 (δ_F = –162.9 ppm) in ^{19}F NMR spectra, and H_3PO_4 in $^{31}P\{^1H\}$ spectra. High-resolution mass spectra (HRMS) were measured with a DFS Thermo scientific instrument (EI, 70 eV).

*4.1.1. Reaction between quinone **1** and PPh_3 in anhydrous C_6H_6 and fixing the formation of betaine **2**.*

PPh_3 (0.493 g, 1.88 mmol) in anhydrous C_6H_6 (1 mL) was added slowly to a stirred solution of quinone **1** (0.500 g, 1.88 mmol) in anhydrous C_6H_6 (20 mL) at room temperature under dried argon. After 30 min, then the reaction mixture changed color from orange to green, the ^{19}F и $^{31}P\{^1H\}$ NMR spectra of solution were recorded under dried argon. ^{19}F NMR (282.36 MHz, C_6D_6 + C_6H_6): –77.6 (br. s, 2F, CF_2), –138.8 and –139.2 [ddd, 1F, $^3J_{FF}$ ~ 20 Hz, $^4J_{FF}$ ~ 10 Hz, $^5J_{FF}$ ~ 13 Hz; $F^{5,8}$], –144.9 and –151.0 [ddd, 1F, $^3J_{FF}$ ~ 20 Hz, $^4J_{FF}$ ~ 10 Hz; $F^{6,7}$] ppm. $^{31}P\{^1H\}$ NMR (121.49 MHz, C_6D_6 + C_6H_6): 16.8 (s) ppm.

*4.1.2. Triphenyl[5,6,7,8-tetrafluoro-1-oxido-4-oxo-3-(phenylimino)-3,4-dihydronaphthalen-2-yl]phosphonium (**4**).*

PPh_3 (0.493 g, 1.88 mmol) in anhydrous C_6H_6 (1 mL) was added slowly to a stirred solution of quinone **1** (0.500 g, 1.88 mmol) in anhydrous C_6H_6 (20 mL) at room temperature under dried argon. After 30 min, then the reaction mixture changed color from orange to green, the ^{19}F и $^{31}P\{^1H\}$ NMR spectra of solution were recorded under dried argon. Then aniline (0.184 g, 1.98 mmol) was slowly added to benzene solution and after 30 min, the NMR spectra of mixture were recorded under dried argon (Scheme 1). The solvent was distilled off, the residue was purified by TLC (Sorbfil, chloroform–hexane, 6:1, R_f = 0.4, 1 time). After double TLC under the same conditions, betaine **4** was obtained as black crystals, (0.394 g, 36%), *m.p.* 286–288 °C. Crystals suitable for XRD analysis were grown from chloroform/hexane (1:4). ^1H NMR (400.13 MHz, $CDCl_3$): δ = 7.75–7.83 (m, 6H, C_6H_5), 7.56–7.63 (m, 3H, C_6H_5), 7.47–7.55 (m, 6H, C_6H_5), 7.1 [tm, 2H, $^3J_{HH}$ = 7.8 Hz, NC_6H_5], 6.9 [tm, 1H, $^3J_{HH}$ = 7.4 Hz, NC_6H_5], 6.1 [d, 2H, $^3J_{HH}$ = 8.3 Hz, NC_6H_5] ppm. $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$): δ = 180.2 [d, $^2J_{CP}$ = 10.2 Hz, C^1], 174.8 [d, $^3J_{CF}$ = 6.4 Hz, C^4], 154.1 [d, $^2J_{CP}$ = 3.5 Hz, C^3], 150.7 (s, NC_6H_5), 146.2 [dm, $^1J_{CF}$ ~ 274 Hz, $C^{5,8}$], 144.8 [dm, $^1J_{CF}$ ~ 262 Hz, $^2J_{CF}$ = 14.5 Hz, $C^{6\text{ or }7}$], 141.8 [dm, $^1J_{CF}$ ~ 260 Hz, $^2J_{CF}$ = 14.6 Hz, $C^{6\text{ or }7}$], 133.5 [d, $^3J_{CP}$ = 10.2 Hz, C_6H_5], 132.3 [d, $^4J_{CP}$ = 2.8 Hz, C_6H_5], 128.8 [d, $^2J_{CP}$ = 12.8 Hz, C_6H_5], 128.4 (s, NC_6H_5), 123.9 [d, $^1J_{CP}$ = 93.2 Hz, $C^{1'}$], 122.3 (s, NC_6H_5), 120.9 [dm, $^2J_{CF}$ = 13.7 Hz, $C_{10}F_4O_2$], 118.9 (s, NC_6H_5), 79.2 [d, $^1J_{CP}$ = 106.2 Hz, C^2] ppm. ^{19}F NMR (282.36 MHz, $CDCl_3$): δ = –138.5, –138.8, –143.6, –149.0, [ddd, 1F, $^{ortho}J_{FF}$ 19.1÷20.5 Hz, $^{meta}J_{FF}$ 7.6÷9.2 Hz, $^{para}J_{FF}$ 14.2÷14.3 Hz] ppm. $^{31}P\{^1H\}$ (121.49 MHz, $CDCl_3$): δ = 15.6 (s) ppm. HRMS: calcd. for $C_{34}H_{20}F_4NO_2P$ [M] 581.1162; found 581.1156; Anal. calcd. for $C_{34}H_{20}F_4NO_2P$: C, 70.23; H, 3.47; N, 2.41; found: C, 70.47; H, 3.51; N, 2.49.

*4.1.3. (3,5-Difluorophenyl)(methyl)phenyl(5,6,7,8-tetrafluoro-3-oxido-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phosphonium (**5**).*

A mixture of quinone **1** (0.158 g, 0.59 mmol), (3,5-difluorophenyl)(methyl)phenylphosphane (0.140 g, 0.59 mmol) and methanol (2.6 mL) was stirred for 72 h at room temperature under dried argon. A precipitate was centrifuged off, washed with methanol (2×0.5 mL) and dried on air to give the title compound **5** (0.037 g, 13%) as bright yellow crystals. After evaporation of the solvent, the dry residue was crystallized from methanol to yield an additional amount (0.070 g) of the product, an overall yield of **5** being 0.107 g (38%), bright yellow crystals, *m.p.* 195–197 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 7.63–7.79 (m, 3H, C_6H_5), 7.51–7.63 (m, 2H, C_6H_5), 7.17 [ddt, 2H, $^{3}J_{HF}$ = 14.0 Hz, $^{3}J_{HP}$ = 5.3 Hz, $^{4}J_{HH}$ = 1.6 Hz, $C_6F_2H_3$], 7.0 [tt, 1H, $^{3}J_{HF}$ = 8.5 Hz, $^{4}J_{HH}$ = 2.0 Hz, $C_6F_2H_3$], 2.54 [d, 3H, $^{2}J_{HP}$ = 13.9 Hz, CH_3] ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, $CDCl_3$): δ = 178.2 (m, $C^{1,4}$), 174.0 [d, $^{2}J_{CP}$ = 3.7 Hz, C^{3}], 163.2 [ddd, $^{1}J_{CF}$ = 254.9 Hz, $^{3}J_{CF}$ = 21.2 Hz, $^{3}J_{CP}$ = 11.6 Hz, $C^{3',5'}$], 147.5 [ddm, $^{1}J_{CF}$ ~ 272 Hz, $^{2}J_{CF}$ ~ 12 Hz, $C^{5\text{ or }8}$], 146.7 [ddm, $^{1}J_{CF}$ ~ 267 Hz, $^{2}J_{CF}$ ~ 11 Hz, $C^{5\text{ or }8}$], 145.3 [dddd, $^{1}J_{CF}$ ~ 264 Hz, $^{2}J_{CF}$ ~ 16 Hz, $^{2}J_{CF}$ ~ 12 Hz, $^{3}J_{CF}$ ~ 3 Hz, $C^{6\text{ or }7}$], 142.8 [dddd, $^{1}J_{CF}$ ~ 262 Hz, $^{2}J_{CF}$ ~ 16 Hz, $^{2}J_{CF}$ ~ 13 Hz, $^{3}J_{CF}$ ~ 3 Hz, $C^{6\text{ or }7}$], 133.8 [d, $^{4}J_{CP}$ = 3.0 Hz, C_6H_5], 132.1 [d, $^{3}J_{CP}$ = 11.0 Hz, C_6H_5], 129.6 [d, $^{2}J_{CP}$ = 13.2 Hz, C_6H_5], 128.9 [dt, $^{1}J_{CP}$ = 93.0 Hz, $^{3}J_{CF}$ = 8.0 Hz, $C^{1'}$], 121.9 [d, $^{1}J_{CP}$ = 92.7 Hz, C_6H_5], 119.0 [dm, $^{2}J_{CF}$ = 11.4 Hz, $C_{10}F_4O_2$], 114.8 [dd, $^{2}J_{CF}$ = 27.7 Hz, $^{2}J_{CP}$ = 11.6 Hz, $C^{2',6'}$], 114.8 [dd, $^{2}J_{CF}$ = 11.3 Hz, $C^{4'}$], 108.8 [ddd, $^{2}J_{CF}{}^{3'\text{ and }CF}{}^{5'}$ = 24.9 Hz, $^{4}J_{CP}$ = 1.3 Hz, $C^{4'}$], 89.1 [d, $^{1}J_{CP}$ = 99.5 Hz, C^{2}], 12.8 [d, $^{1}J_{CP}$ = 60.2 Hz, CH_3] ppm. ^{19}F NMR (282.36 MHz, $CDCl_3$): δ = –105.8 (m, 2F, $C_6F_2H_3$), –137.9, –140.6, –144.4, –149.1, [ddd, 1F, $^{ortho}J_{FF}$ 19.4÷20.0 Hz, $^{meta}J_{FF}$ 9.4÷11.3 Hz, $^{para}J_{FF}$ 13.4÷13.5 Hz] ppm. $^{31}P\{^{1}H\}$ (121.49 MHz, $CDCl_3$): δ = 14.6 [t, $^{4}J_{PF}$ 7.0 Hz] ppm. HRMS: calcd. for $C_{23}H_{11}F_6O_3P$ [M] 480.0345; found 480.0346; Anal. calcd. for $C_{23}H_{11}F_6O_3P$: C, 57.52; H, 2.31; F, 23.73; P, 6.45; found: C, 57.58; H, 2.52; F, 23.78; P, 6.30.

*4.1.4. (2,5-Difluorophenyl)(methyl)phenyl(5,6,7,8-tetrafluoro-3-oxido-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phosphonium (**6**)*

A mixture of quinone **1** (0.088 g, 0.33 mmol), (2,5-difluorophenyl)(methyl)phenylphosphane (0.078 g, 0.33 mmol) and DMSO (1.0 mL) was stirred for 7 h at room temperature under dried argon and analyzed by ^{19}F NMR and $^{31}P\{^{1}H\}$ NMR (Scheme 2). To solution was quickly added (2,5-difluorophenyl)(methyl)phenylphosphane (0.016 g, 0.07 mmol) in DMSO (1.0 mL). The resulting mixture was stirred for 1 h at room temperature under dried argon. Water (~8 mL) was added; a precipitate was centrifuged off, washed with water (16 mL) and dried on air. The dry residue was crystallized from methanol to yield the title compound **6** (0.107 g, 67%) as bright yellow crystals, *m.p.*213–215 °C. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 7.63–7.75 (m, 3H, C_6H_5), 7.52–7.61 (m, 2H, C_6H_5), 7.24–7.33 (m, 1H, $C_6F_2H_3$), 7.12–7.23 (m, 1H, $C_6F_2H_3$), 6.87–7.00 (m,

1H, $C_6F_2H_3$), 2.62 [dd, 3H, $^2J_{HP}$ = 14.4 Hz, $^5J_{HF}$ = 1.8 Hz, CH_3] ppm. $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$): δ = 178.1 (m, $C^{1,4}$), 173.7 [d, $^2J_{CP}$ = 3.8 Hz, C^3], 159.5 [dd, $^1J_{CF}$ = 247.4 Hz, $^3J_{CP}$ = 2.6 Hz, $C^{5'}$], 158.6 [ddd, $^1J_{CF}$ = 247.6 Hz, $^2J_{CP}$ = 16.0 Hz, $^4J_{CF}$ = 2.2 Hz, $C^{2'}$], 147.5 [dd, $^1J_{CF}$ ~ 268 Hz, $^2J_{CF}$ = 10.1 Hz, $C^{5 \text{ or } 8}$], 146.7 [dd, $^1J_{CF}$ ~ 268 Hz, $^2J_{CF}$ = 10.7 Hz, $C^{5 \text{ or } 8}$], 144.8 [dddd, $^1J_{CF}$ ~ 264 Hz, $^2J_{CF}$ ~ 17 Hz, $^2J_{CF}$ ~ 13 Hz, $^3J_{CF}$ ~ 3 Hz $C^{6 \text{ or } 7}$], 142.8 [dddd, $^1J_{CF}$ ~ 262 Hz, $^2J_{CF}$ ~ 16 Hz, $^2J_{CF}$ ~ 13 Hz, $^3J_{CF}$ ~ 3 Hz $C^{6 \text{ or } 7}$], 133.7 [d, $^4J_{CP}$ = 3.1 Hz, C_6H_5], 131.8 [d, $^3J_{CP}$ = 11.2 Hz, C_6H_5], 129.6 [d, $^2J_{CP}$ = 13.4 Hz, C_6H_5], 122.5 [ddd, $^2J_{CF}$ = 24.2 Hz, $^3J_{CF}$ = 9.2 Hz, $^4J_{CP}$ = 2.5 Hz, $C^{4'}$], 121.8 [d, $^1J_{CP}$ = 94.1 Hz, $C^{1'}$], 121.0 [ddd, $^2J_{CF}$ = 26.6 Hz, $^3J_{CF}$ = 8.3 Hz, $^3J_{CP}$ = 3.5 Hz, $C^{3'}$], 119.1 [dm, $^2J_{CF}$ = 11.5 Hz, $C_{10}F_4O_2$], 118.2 [dt, $^2J_{CF}$ = 25.3 Hz, $^3J_{CF}$ ~ $^2J_{CP}$ = 7.5 Hz, $C^{6'}$], 116.5 (s, C^3), 113.9 [ddd, $^1J_{CP}$ = 91.2 Hz, $^2J_{CF}$ = 19.6 Hz, $^3J_{CF}$ = 6.8 Hz, $C^{1'}$], 88.6 [d, $^1J_{CP}$ = 101.5 Hz, C^2], 11.6 [dd, $^1J_{CP}$ = 61.0 Hz, $^4J_{CF}$ = 4.2 Hz, CH_3] ppm. ^{19}F NMR (282.36 MHz, $CDCl_3$): δ = –108.7 (m, $C_6F_2H_3$), –116.7 (m, $C_6F_2H_3$), –138.6, –141.4, –145.2, –149.9, [ddd, 1F, $^{ortho}J_{FF}$ 19.5÷20.0 Hz, $^{meta}J_{FF}$ 9.3÷11.6 Hz, $^{para}J_{FF}$ 13.1÷13.4 Hz] ppm. $^{31}P\{^1H\}$ (121.49 MHz, $CDCl_3$): δ = 11.2 [d, $^3J_{PF}$ = 3.6 Hz] ppm. HRMS: calcd. for $C_{23}H_{11}F_6O_3P$ [M] 480.0345; found 480.0353; Anal. calcd. for $C_{23}H_{11}F_6O_3P$: C, 57.52; H, 2.31; F, 23.73; P, 6.45; found: C, 57.47; H, 2.57; F, 23.77; P, 6.38.

*4.1.5. (3,5-Difluorophenyl)diphenyl(5,6,7,8-tetrafluoro-3-oxido-1,4-dioxo-1,4-dihydronaphthalen-2-yl)phosphonium (**7**).*

A mixture of quinone **1** (0.06 g, 0.23 mmol), (3,5-difluorophenyl)diphenylphosphane (0.081 g, 0.27 mmol) and DMSO (1.6 mL) was stirred for 1 h at room temperature under dried argon and analyzed by ^{19}F NMR and $^{31}P\{^1H\}$ NMR (Scheme 2). Water (~8 mL) was added; a precipitate was centrifuged off, washed with water (16 mL) and dried on air. The dry residue was crystallized from methanol to yield the title compound **7** (0.07 g, 57%) as bright yellow crystals, *m.p.*231–233 °C. ^1H NMR (300.13 MHz, $CDCl_3$): δ = 7.62–7.73 (m, 6H, C_6H_5), 7.50–7.59 (m, 4H, C_6H_5), 7.15 [ddt, 2H, $^3J_{HF}$ = 13.6 Hz, $^3J_{HP}$ = 5.4 Hz, $^4J_{HH}$ = 1.8 Hz, $C_6F_2H_3$], 7.02 [tt, 1H, $^3J_{HF}$ = 8.5 Hz, $^4J_{HH}$ = 2.1 Hz, $C_6F_2H_3$] ppm. $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$): δ = 178.3 (m, $C^{1,4}$), 173.7 [d, $^2J_{CP}$ = 3.2 Hz, C^3], 162.8 [ddd, $^1J_{CF}$ = 254.3 Hz, $^3J_{CF}$ = 21.0 Hz, $^3J_{CP}$ = 11.6 Hz, $C^{3',5'}$], 147.4 [dddd, $^1J_{CF}$ ~ 271 Hz, $^2J_{CF}$ ~ 11 Hz, $^3J_{CF}$ ~ 4 Hz, $^4J_{CF}$ ~ 2 Hz, $C^{5 \text{ or } 8}$], 146.5 [dddd, $^1J_{CF}$ ~ 270 Hz, $^2J_{CF}$ ~ 11 Hz, $^3J_{CF}$ ~ 3 Hz, $^4J_{CF}$ ~ 2 Hz, $C^{5 \text{ or } 8}$], 145.3 [dddd, $^1J_{CF}$ ~ 230 Hz, $^2J_{CF}$ ~ 16 Hz, $^2J_{CF}$ ~ 12 Hz, $^3J_{CF}$ ~ 3 Hz, $C^{6 \text{ or } 7}$], 142.7 [dddd, $^1J_{CF}$ ~ 230 Hz, $^2J_{CF}$ ~ 16 Hz, $^2J_{CF}$ ~ 12 Hz, $^3J_{CF}$ ~ 3 Hz, $C^{6 \text{ or } 7}$], 133.5 [d, $^4J_{CP}$ = 3.4 Hz, C_6H_5], 133.5 [d, $^3J_{CP}$ = 11.0 Hz, C_6H_5], 129.4 [d, $^2J_{CP}$ = 13.2 Hz, C_6H_5], 128.0 [dt, $^1J_{CP}$ = 93.1 Hz, $^3J_{CF}$ = 8.1 Hz, $C^{1'}$], 121.1 [d, $^1J_{CP}$ = 93.4 Hz, $C^{1'}$], 119.3 [dm, $^2J_{CF}$ = 11.0 Hz, $C_{10}F_4O_2$], 116.3 [dd, $^2J_{CF}$ = 28.0 Hz, $^2J_{CP}$ = 11.1 Hz, $C^{2',6'}$], 108.6 [ddd, $^2J_{CF^{3'} \text{ and } CF^{5'}}$ = 25.0 Hz, $^4J_{CP}$ = 1.7 Hz, $C^{4'}$], 88.6 [d, $^1J_{CP}$ = 100.1 Hz, C^2] ppm. ^{19}F NMR (282.36 MHz, $CDCl_3$): δ = –106.8 (m, 2F, $C_6F_2H_3$), –138.4, –140.7, –145.0, –149.8, [ddd, 1F, $^{ortho}J_{FF}$ 19.4÷20.1 Hz, $^{meta}J_{FF}$ 9.1÷11.2 Hz, $^{para}J_{FF}$ 13.6÷13.7 Hz]

ppm. $^{31}P\{^{1}H\}$ (121.49 MHz, $CDCl_3$): δ = 15.4 [t, $^{4}J_{PF}$ = 7.0 Hz] ppm. HRMS: calcd. for $C_{28}H_{13}F_6O_3P$ [M] 542.0501; found 542.0494; Anal. calcd. for $C_{28}H_{13}F_6O_3P$: C, 62.01; H, 2.42; F, 21.02; P, 5.71; found: C, 62.36; H, 2.68; F, 20.83; P, 5.75.

4.2. Crystallographic Study

XRD data for **4** were obtained on a Bruker Kappa Apex II CCD diffractometer using *φ, ω* scans of narrow (0.5°) frames with Mo Kα radiation (λ = 0.71073 Å) and a graphite monochromator. The structures were solved by direct methods and refined by full-matrix least-squares method against all F^2 in anisotropic approximation using the *SHELX-97* programs set [29]. The H atoms positions were calculated with the riding model. Absorption corrections were applied empirically using *SADABS* programs. Crystals of compound **4** are triclinic, space group *P-1*, *a* = 10.463(1), *b* = 10.519(1), *c* = 13.224(1) Å, α = 82.623(5), *β* = 69.266(5), γ = 79.776(5) °, *V* = 1336.1(3) $Å^3$, *Z* = 2, $C_{34}H_{20}F_4NO_2P$, D_c = 1.445 г/см^3, *μ* = 0.165 mm^{-1}, F(0 0 0) = 596, independent reflections 4758, for 1722 reflections with I > 2σ: R = 0.0534, wR_2 = 0.1431, S = 1.06 (for all reflections: R = 0.0715, wR_2 = 0.1743). The obtained crystal structures were analyzed for short contacts between non-bonded atoms using the *PLATON* program [30]. Tables listing detailed crystallographic data, atomic positional parameters, and bond lengths and angles are available as CCDC 1452176 from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.3. Biological activity

4.3.1. Determination of mutagenisity of compounds

In the Ames test, the histidine-dependent strain of *S. typhimurium* TA102 having a mutation at the histidine operon was used. [27]. The mutagenic activities of compounds **4–7** were analyzed by the standard method (without metabolic activation) [27] according to [28]. TA102 (a liquid culture) was prepared by growth of cells (from a frozen stock) during 16-h at 37 °C in LB medium containing penicillin. The cells obtained were plated on minimal glucose agar containing histidine and antibiotics at the density sufficient to obtain isolated colonies. A separate bacterial colony was inoculated into 5 mL LB medium supplemented with tetracycline (2 μg/mL) and ampicillin (50 μg/mL); the growth of cells was performed with shaking (130 rpm) for 15 h at 37°C. The Ames test was performed using the double-layer method according to [27]. To the overnight culture of bacteria (100 μl) one of the tested compounds was added (in different concentrations). In some experiments 0.1 ml of 63 mM H_2O_2 (final concentration 3 mM), were mixed with 2 mL of liquid 0.6% top agar at 42° C. This mixture was poured onto plates containing a minimal medium supplemented with 3% agar and 0.2% glucose; it was uniformly distributed on the surface of the solid agar. The plates were incubated at 37°C for 48 h, and the revertants were counted. The cells

incubated with H_2O_2 in the absence of any compounds were served as positive controls, while cells incubated in the absence of antioxidants and H_2O_2 was used as negative controls for mutation induction. The data of experiments are expressed as mean ± S.E. corresponding to at least 3 independent experiments.

4.3.2. Cytotoxicity assays

Tumor cell lines from human mammary adenocarcinoma MCF-7, human myeloma RPMI 8226, hepatocellular carcinoma epithelial tumor cells HepG2, as well as normal Chinese hamster Ag 17 cells (AG) fibroblast cell line, and normal mouse fibroblasts (LMTK) were used. The cells (~$2x10^3$ cells per well) were incubated at 37ºC for 24 h using RPMI 1640 or IMDM medium (5% CO_2) and then compounds **4–7** were added as in [31]. The relative amount of live cells (after 72 h of cell incubation) was estimated using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide according to a standard MTT-colorimetric test [31]. The compound concentration causing 50% cell growth inhibition (IC_{50}) was determined. The results were presented as mean ± S.E. of three independent experiments.

4.4. Docking studies

The docking analysis of molecules was carried out using Autodock Vina [32]. Ligand molecules were sketched in 3D format using OpenBabel module of PyRx GUI. Merck molecular force field minimization algorithm [33] was used to produce low-energy conformers. Cdc25B model (PDB ID 1QB0, 1.91 Å resolution) and phosphonium betaines were prepared by adding hydrogens, adding missing residues, and converting to PDBQT format. All crystallographic water molecules and other chemical components were omitted, the right bond orders as well as charges and atom types were assigned. The grid coordinates for molecular docking was chosen according to Cdc25B catalytic pockets study [25]. Molecules were docked using Vina with exhaustiveness grade 8, with up to 9 poses saved per molecule. The docking procedure was carried out for the unchanged conformation of the receptor and flexible ligand molecules. The lowest energy modes were selected and the ligands interactions with binding site were determined. The UCSF Chimera 1.10 and Accelrys Discovery Studio Visualizer 2016 were utilized for docking and interaction visualization.

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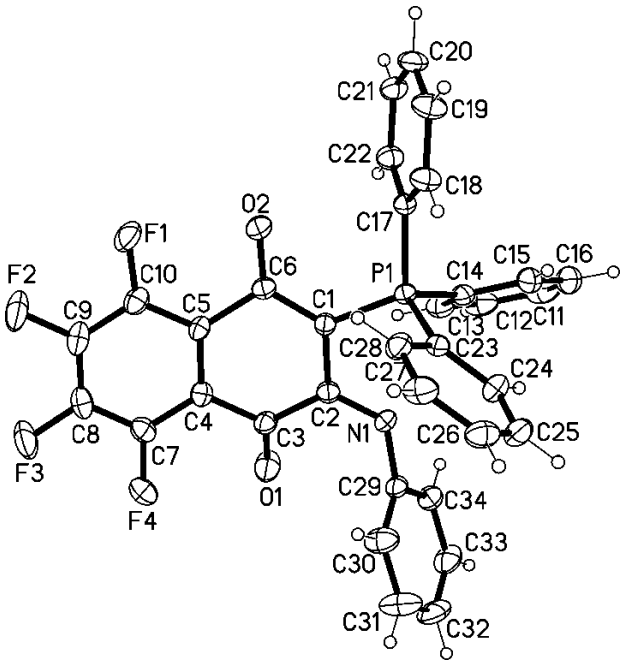


Fig. 1. The X-ray molecular structure of compound **4**. Selected bond lengths (Å): P1–C1 1.767(3), N1–C2 1.294(4), O1–C3 1.203(4), O2–C6 1.231(4), C1–C6 1.420(4), C1–C2 1.436(4), C2–C3 1.504(4), C3–C4 1.488(4), C4–C5 1.408(4), and C5–C6 1.519(4).

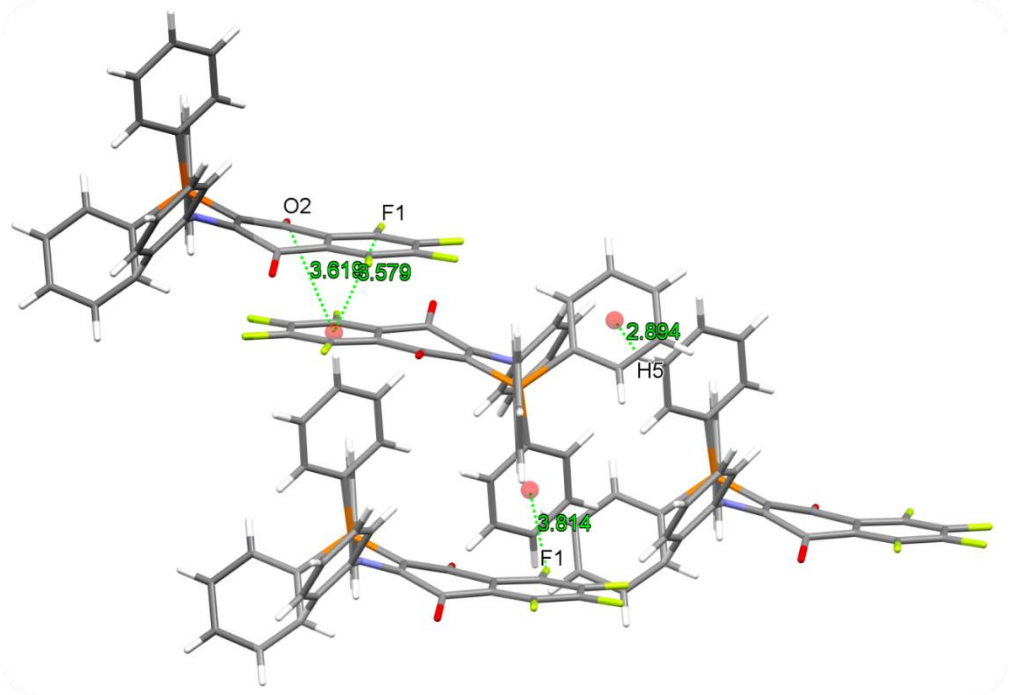


Fig. 2. A part of packing and atom...π interactions in the crystal structure of **4** [Centroid...F1 3.579(3) and 3.814(3), Centroid....O2 3.619(4), and Centroid...H5 2.89 A].

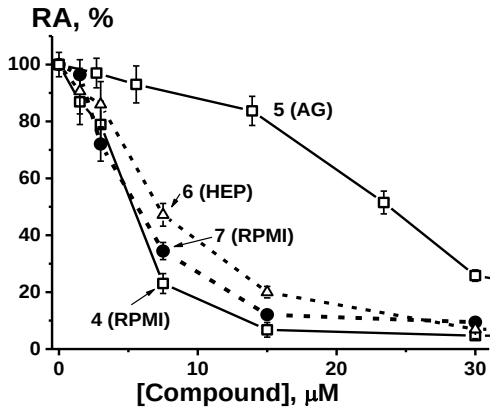
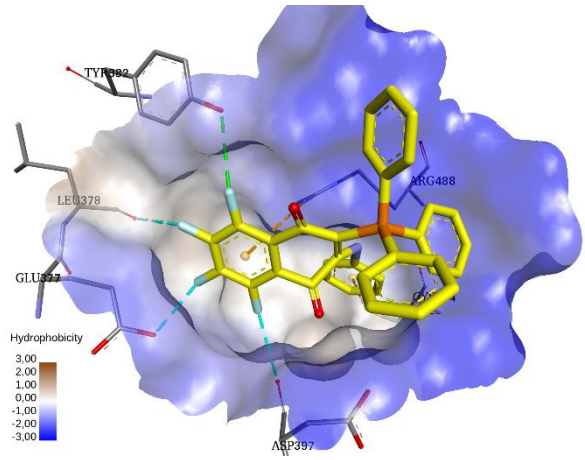
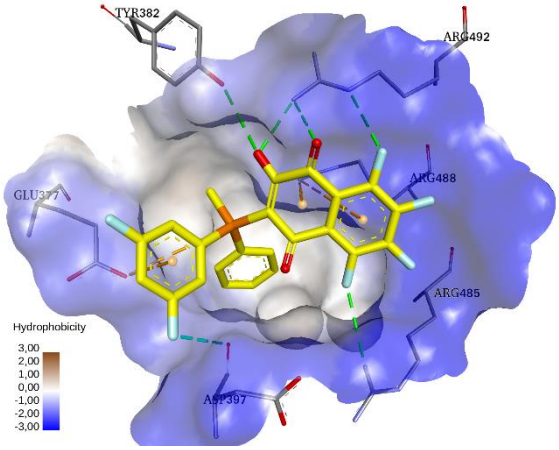


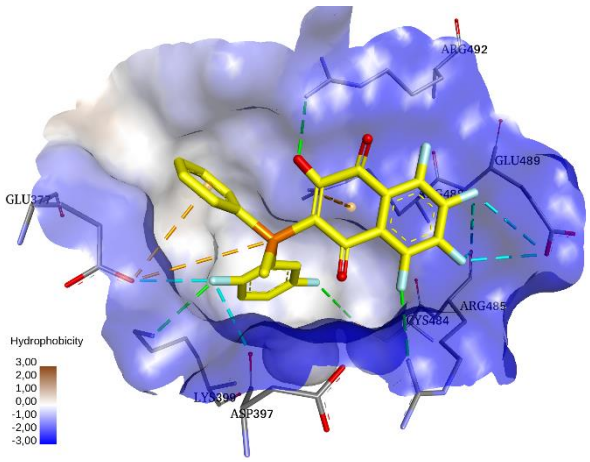
Fig. 3. Effects of fluorinated compounds **4–7** (relative activity, RA) on growth of various cells.



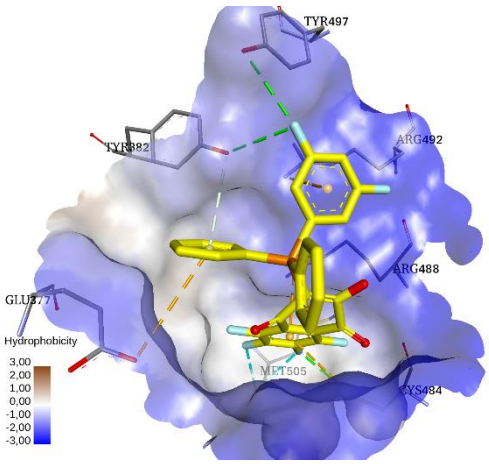
4 (–7.9 kcal/mol)



5 (–8.5 kcal/mol)



6 (–8.5 kcal/mol)



7 (–8.4 kcal/mol)

Fig. 4. The best binding modes of phosphonium betaines predicted by means of the AutoDock Vina scoring function. The noncovalent interactions are indicated by dashed lines: green, hydrogen bonds; blue, fluorine bonds; orange, electrostatic interactions. The hydrophobic intensity of the binding site ranges from −3.00 (the least hydrophobic region: blue shading) to 3.00 (a highly hydrophobic region: brown shading).

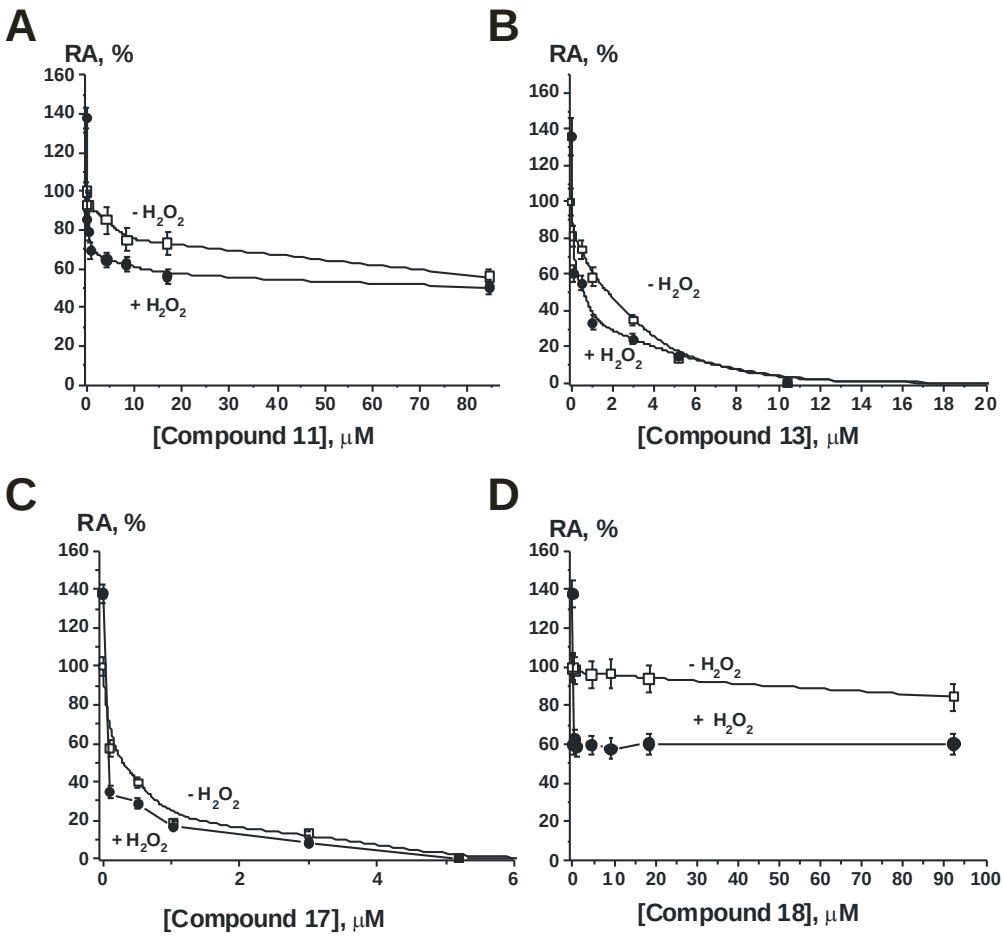
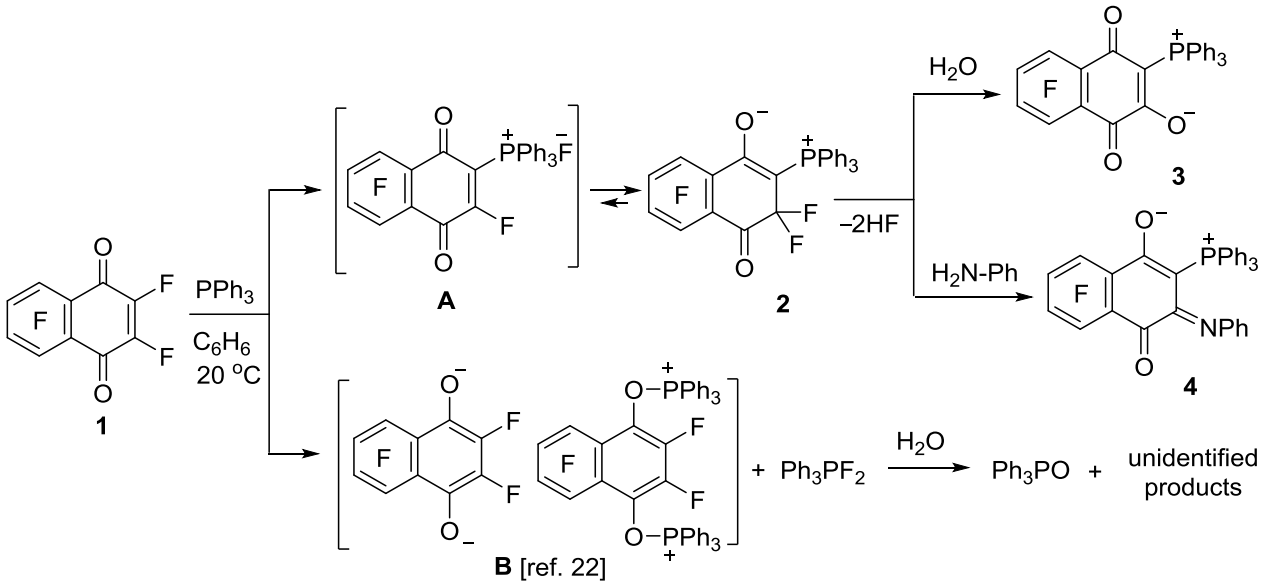
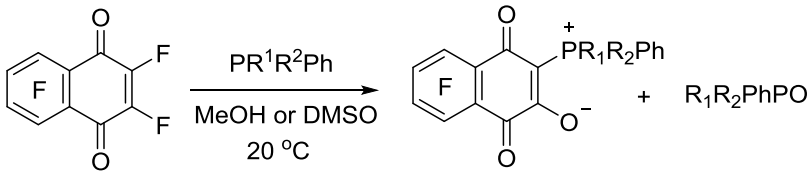


Fig. 5. Analysis of antioxidant and mutagenic effects of compounds **4** (A), **5** (B), **6** (C), and **7** (D) by the standard Ames test using the *S. typhimurium* strain TA102 in the absence (□) or presence (■) of 3 mM H_2O_2 (A–D). The number of revertants without H_2O_2 was set to 100%.



Scheme 1. Reaction of hexafluoro-1,4-naphthoquinone **1** with PPh_3 in dried C_6H_6 followed by hydrolysis or treatment with aniline.



R^1 = Me, R^2 = 3,5-$F_2C_6H_3$ **5** (38%)

R^1 = Me, R^2 = 2,5-$F_2C_6H_3$ **6** (67%)

R^1 = Ph, R^2 = 3,5-$F_2C_6H_3$ **7** (57%)

Scheme 2. Reactions of hexafluoro-1,4-naphthoquinone **1** with fluorinated PR^1R^2Ph.

Table 1

Cytotoxicity (IC_{50}) of fluorinated derivatives of phosphonium betaines **4–7**.

Compound	IC_{50}, μM					Average ratio of 1-3 and 4-5
	MCF-7	RPMI 6228	HEP	LMTK	AG-17	
	1	2	3	4	5	
4	5.2±0.4	5.8±0.3	4.5±0.3	7.6±0.6	19.8±0.7	1.3–4.4
5	4.6±0.3	6.4±0.4	7.1±0.5	7.7±0.5	23.0±1.6	1.1–5.0
6	5.5±0.4	7.9±0.5	7.9±0.4	10.4±0.7	50.0±3.0	1.3–9.1
7	5.7±0.3	5.5±0.4	7.6±0.9	7.6±0.6	19.8±1.1	1.0–3.6

*Mean ± SD from three independent experiments.

Table 2

IC_{50} characterizing suppression of spontaneous and H_2O_2-induced mutations by polyfluorinated derivatives of 1,4-naphthoquinone.

Compound	IC_{50}, μM (% of inhibition at 50 μg/ml)*		
	Suppression of spontaneous mutagenesis (from 100 to 50%)	Suppression of H_2O_2-induced and spontaneous mutagenesis, %	
		From 137.7 to 100%	From 100 to 50%
4	(44.4 ±3.5 %)**	0.09±0.006	86.0±7.0
5	1.8±0.09	0.057±0.004	0.62±0.05
6	0.26±0.03	0.04±0.003	0.09±0.005
7	(15.5 ±1.2 %)	0.05±0.004	(40.0 ±3.0 %)

*Mean ± SD from four independent experiments.

The percentage of inhibition at 50 μg/ml; compound **4: 86 μM, compound **7**: 92.2 μM.