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Versatile approach to naphthoquinone phosphonium salts and evaluation of their biological activity

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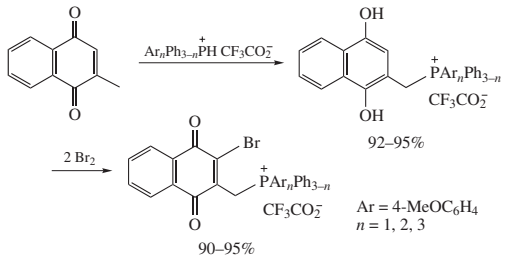
A new efficient method for the synthesis of 1,4-dihydroxy-2-naphthylmethyl- and 1,4-dioxo-2(1*H*,4*H*)-2-naphthylmethylphosphonium salts by reactions of 2-methyl-1,4-naphthoquinone with appropriate P–H-phosphonium trifluoroacetates has been suggested. The reactions were carried out under mild conditions and provided high yields of target compounds. Representative reaction products have been found to possess moderate antimicrobial and antitumor activity.

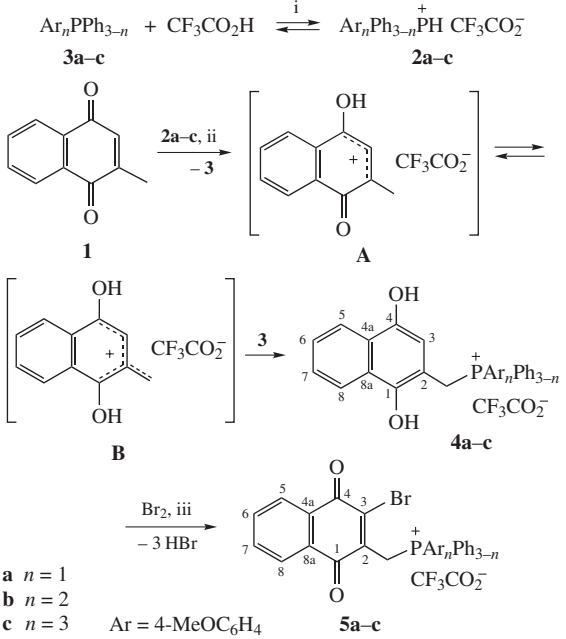


Phosphonium salts are extensively used in various areas of chemistry, biology and pharmacology.[1] In particular, their application is related to mitochondria as a target for treatment of oncological,[2–4] cardiovascular and neurological diseases. The lipophilic arylphosphonium moieties are typically incorporated into the structure of pharmacologically active compounds for their efficient delivery to mitochondria.[5,6] The delocalization of the phosphorus atom positive charge by aryl substituents results in lowering the activation energy required for the penetration through lipid membrane. As a consequence, phosphonium salts are easily accumulated in mitochondria, which have a large negative membrane potential.[7,8] In general, tumor cells are characterized by a greater negative potential across the inner mitochondrial membrane compared with normal cells, therefore phosphonium salts are considered as promising agents for diagnostics and treatment of cancer.[9,10]

We developed an approach to the synthesis of phosphonium salts, based on the addition of H-phosphonium salts to epoxides,[11] 1,4-benzo- and 1,4-naphthoquinone.[12,13] It allows one to obtain functionally substituted phosphonium salts without use of tertiary phosphines. The starting H-phosphonium salts with a labile phosphorus–hydrogen bond can be prepared by reactions of trialkyl(aryl)phosphines with different acids.[14–16]

As we demonstrated, the outcome of quinones phosphorylation depends not only on their structure, but also on the counterion in the H-phosphonium salt used. For example, the reaction of 2-methyl-1,4-naphthoquinone (menadione) **1** with triphenylphosphonium trifluoromethylsulfonate results in incorporation of a phosphorus atom to 3-position of the naphthalene moiety.[13] Contrary to that, in this work we have shown that various phosphonium trifluoroacetates **2a–c**, obtained from the corresponding phosphines **3a–c** and trifluoroacetic acid (TFA), react with quinone **1** to give a different synthetic result, namely new functionally substituted phosphonium salts **4**,[†] in which the phosphorus atom is bound to the side chain of the starting quinone rather than to the aromatic moiety (Scheme 1). The reaction occurs under mild conditions and leads to compounds **4a–c** in high yields.

This difference in the reaction products may be explained by the lower protonating ability of TFA in comparison with trifluoromethanesulfonic acid used previously. In the former case, the resulting H-phosphonium salts **2a–c** are apparently in equilibrium with the corresponding phosphines **3a–c** and free TFA, which



Scheme 1 *Reagents and conditions*: i, CH_2Cl_2, room temperature, 1 h; ii, CH_2Cl_2, room temperature, 24 h; iii, CH_2Cl_2, room temperature, 2 h.

† *Synthesis of H-phosphonium salts* **2a–c** *(general procedure)*. An equimolar amount of TFA was added dropwise to a solution of the corresponding phosphine **3** (1 g) in CH_2Cl_2 (5.5 ml) with continuous stirring and bubbling with dry argon. The mixture was stirred for 1 h. The resulting triarylphosphonium trifluoroacetate **2** was used in the subsequent step without isolation.

Table 1 Antimicrobial activity of compounds **4a–c**.[a]

Compound	Bacteriostatic and fungistatic activity, MIC/μM						
	S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*A. niger*	*T. mentagrophytes*	*C. albicans*
4a	216.0±18.5	216.1±16.8	>500	>500	>500	>500	>500
4b	205.4±17.7	205.2±17.5	>500	>500	>500	>500	>500
4c	97.7±8.4	195.4±15.4	>500	>500	>500	>500	>500
Chloramphenicol	193±17	193±17	386±35	–			
Ketoconazole						7.6±0.6	7.6±0.7
	Bactericidal and fungicidal activity, MBC and MFC/μM						
4a	216.0±17.9	432.2±43.1	>500	>500	>500	>500	>500
4b	205.3±17.6	410.7±34.9	>500	>500	>500	>500	>500
4c	97.9±8.6	>500	>500	>500	>500	>500	>500

[a] MIC is minimum inhibitory concentration, MBC is minimum bactericidal concentration, MFC is minimum fungicidal concentration.

is capable of protonating quinone **1** with intermediate formation of structure **A** (see Scheme 1). The subsequent proton transfer from methyl group to the oxygen atom leads to the tautomeric form **B**. The following attack of the phosphorus atom in phosphine **3** on the methylene group of the form **B** results in (2-naphthylmethyl)phosphonium salt **4**.

The structure of phosphonium salts **4a–c** was confirmed by the presence of signals corresponding to P^+–CH_2 moiety in the ^1H and ^{13}C NMR spectra. In fact, in the ^1H NMR spectrum of phosphonium salt **4a**, the methylene group protons appear as a doublet at δ 5.02 ppm with $^2J_{HP}$ 14.9 Hz. In the upfield region, only the singlet of MeO group is present at δ 3.87 ppm. In the ^{13}C-{^1H} NMR spectrum, the CH_2 carbon resonates as a doublet at δ_C 25.06 ppm with $^1J_{PC}$ 48.4 Hz. Conversely, the C^3 carbon of the naphthalene moiety remains unaffected in the chemical reaction and therefore resonates as a doublet at δ_C 110.02 ppm with $^3J_{PC}$ 3.7 Hz and with an additional splitting in the ^{13}C NMR spectrum, which is characterized by $^1J_{HC}$ 158.5 Hz.

Next, we treated phosphonium salts **4a–c** with excess bromine under mild conditions. This reaction resulted in the oxidation of 1,4-dihydronaphthalene moiety to 1,4-naphthoquinone one.‡ The spectral data for the isolated products **5a–c** indicates that, in addition to the oxidation process, incorporation of a bromine atom to the naphthalene 3-position occurs (see Scheme 1, step iii).

The formation of quinone structure **5** is followed by a change in color of the reaction mixture from light yellow to bright orange. The IR spectra of the isolated products **5a–c** contain intense absorption bands in the range 1672–1676 cm^{-1}, corresponding to the stretching vibration of conjugated carbonyl group of the quinone moiety. In the ^{13}C NMR spectra, the *ipso* carbon atoms of the quinone moieties resonate in the most downfield region at δ_C 175–180 ppm. In the ^{13}C-{1H} NMR spectra, the carbon atoms of the methylene groups resonate at ~30 ppm as doublets with $^1J_{PC}$ ~50 Hz, which indicates that the CH_2–P^+ moiety is preserved. Conversely, the reaction affects the signal of C^3 in the naphthalene moiety with a considerable downfield shift (δ_C ~144 ppm) and this signal is observed in the ^{13}C-{^1H} NMR spectra as a doublet with a small value of the $^3J_{PC}$ constant, the signal multiplicity being the same in the ^{13}C NMR spectra. This confirms the absence of proton at the C^3 atom and agrees well with the incorporation of bromine atom at 3-position of the naphthalene moiety. The downfield regions of ^{13}C-{^1H} spectra for compounds **4b** and **5b** are demonstrated in Figure 1.

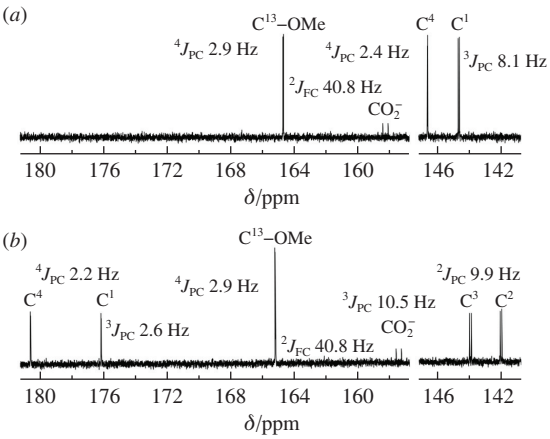


Figure 1 Low field regions of ^{13}C-{^1H} NMR spectra (100.6 MHz, $CDCl_3$) of phosphonium salts: (*a*) **4b** and (*b*) **5b**.

We estimated the *in vitro* bacteriostatic, antibacterial, fungistatic and antifungal activity of phosphonium salts **4a–c** in the concentration range 97.7–432.2 μmol dm^{-3} (Table 1).§ Compounds **4a–c** were found to be active selectively against Gram-positive bacteria *S. aureus* and *B. cereus*, and the salt **4c** had the highest activity with better MIC value towards *S. aureus* compared to chloramphenicol. None of the tested compounds were active against Gram-negative bacteria at the concentration range investigated.

Hemolytic activity of phosphonium salts **4a–c** against human erythrocytes was tested in the antimicrobial MIC and MBC ranges (Table 2).[19] The compounds demonstrated moderate effect with

(1,4-Dihydroxy-2-naphthylmethyl)bis(4-methoxyphenyl)(phenyl)-phosphonium trifluoroacetate **4b**. A solution of 2-methyl-1,4-naphthoquinone **1** (0.18 g, 1.03 mmol) in CH_2Cl_2 (5 ml) was added dropwise to a solution of phosphonium salt **2b** (0.42 g. 1.03 mmol) in CH_2Cl_2 (10 ml) with continuous stirring, cooling in a water bath and bubbling with dry argon. The reaction mixture was stirred for 0.5 h. After 24 h of standing, the red mixture was concentrated *in vacuo* (14 Torr) to give a pink precipitate of compound **4b**, which was purified by recrystallization from acetone–diethyl ether (1 : 10). Yield 0.6 g (95%), mp 200–203 °C.

‡ *[3-Bromo-1,4-dioxo-2(1*H*,4*H*)-naphthylmethyl]bis(4-methoxyphenyl)-(phenyl)phosphonium trifluoroacetate* **5b**. An excess of bromine (0.04 ml, 0.74 mmol) was added with continuous stirring to a solution of phosphonium salt **4b** (0.26 g, 0.37 mmol) in CH_2Cl_2 (10 ml). After 2 h, the evolution of hydrogen bromide ceased (litmus control) and the orange reaction mixture was concentrated *in vacuo* (14 Torr). The precipitate formed was purified by recrystallization from diethyl ether (10 ml). The yellow-orange product obtained was filtered off and dried *in vacuo* (14 Torr). Yield 0.45 g (90%), mp 80–82 °C.

§ The bacteriostatic and fungistatic properties were investigated by serial dilutions in liquid growth medium[17] according to known procedures.[18] *Staphylococcus aureus* 209 P and *Bacillus cereus* NCTC 8035 as representatives of Gram-positive bacteria, *Escherichia coli* CDC F-50 and *Pseudomonas aeruginosa* ATCC 9027 as representatives of Gram-negative bacteria as well as fungi *Aspergillus niger* VKM F-1119, *Trichophyton mentagrophytes var. gypseum* 1773 and *Candida albicans* 855-653 were used as test microorganisms. The experiments were performed in triplicate.

Table 2 Hemolytic activity of compounds **4a–c**.

Compound	Concentration/μM	Hemolysis (%)
4a	432.2±43.1	7.0±0.6
	216.0±18.5	2.6±0.2
	108.0±9.2	2.5±0.1
	54.2±4.6	0.0
4b	410.7±34.9	2.3±0.1
	205.3±17.6	0.90±0.07
	102.7±8.8	0.0
	51.3±4.6	0.0
4c	390.8±30.8	12.3±1.2
	195.4±15.4	12.0±1.1
	97.7±7.7	9.0±0.7
	48.9±3.9	1.9±0.2

Table 3 The cytotoxicity of phosphonium salts **4a–c**.

Compound	IC_{50}/μM, tumor cell lines				IC_{50}/μM, normal cell lines	
	M-HeLa	MCF7	A-549	PC3	Chang liver	WI-38
4a	18.0±1.4	13.0±1.1	>100	16.0±1.3	28.0±2.2	25.0±2.3
4b	13.2±1.2	10.0±0.8	>100	15.0±1.2	28.0±2.4	14.0±1.1
4c	16.0±1.2	11.0±0.9	>100	13.0±1.0	25.0±2.2	33.0±2.7
Doxorubicin	3.0±0.2	3.0±0.1	3.0±0.3	2.6±0.2	3.0±0.2	1.3±0.1

the maximum degree of hemolysis of ~12% at the highest concentrations tested.

Compounds **4a–c** were also investigated for cytotoxicity to normal and tumor human cell lines (Table 3).¶ The phosphonium salts demonstrated cytotoxic effect to tumor cells M-HeLa, MCF7 and PC3 with the IC_{50} range of 10–18 μM and were less toxic to normal Chang liver and WI-38 cells, which is an indicator used in development of new antitumor agents. The phosphonium salts **4a–c** had no cytotoxic activity for tumor cell line A-549.

In summary, we have suggested a mild and efficient method for the synthesis of functionally substituted methylphosphonium salts containing 1,4-dioxo- and 1,4-dihydroxynaphthalene moieties at the methyl group. The method is based on the reaction of 2-methyl-1,4-naphthoquinone with H-phosphonium salts followed by bromination.

¶ The cytotoxic effect was estimated by examination of living cells using a Cytell Cell Imaging multifunctional system (GE Helthcare Life Science, Sweden) equipped with a Cell Viability BioApp application, which provides an accurate counting of the number of cells and estimation of their viability based on fluorescence intensity.[20] The tumor cell cultures used included M-HeLa clone 11 (human cervix carcinoma), MCF7 (human breast carcinoma, pleural fluid), A-549 (human lung adenocarcinoma) and PC-3 (human prostate adenocarcinoma). Normal cell lines included WI-38 (human lung fibroblasts) and Chang liver (human hepatic cells). The cell lines were obtained from collections of the Institute of Cytology of the Russian Academy of Sciences, St. Petersburg, and Institute of Virology of the Russian Academy of Medical Sciences, Moscow.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.07.027.

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