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Synthesis of new *p*-quinone methide containing morpholine fragment: access to (diarylmethyl)phosphonamidates with antitumor activity

Elmira M. Gibadullina,*[a] Tran H. B. Nguyen,[b] Thu T. Nguyen,[b] Anna G. Strelnik,[a] Alexandra D. Voloshina,[a] Anna P. Lyubina,[a] Sumbel K. Amerhanova[a] and Alexander R. Burilov[a]

[a] *A. E. Arbuzov Institute of Organic and Physical Chemistry, FRC Kazan Scientific Center of the Russian Academy of Sciences, 420088 Kazan, Russian Federation. E-mail: elmirak_1978@mail.ru*

[b] *Kazan National Research Technological University, 420015 Kazan, Russian Federation*

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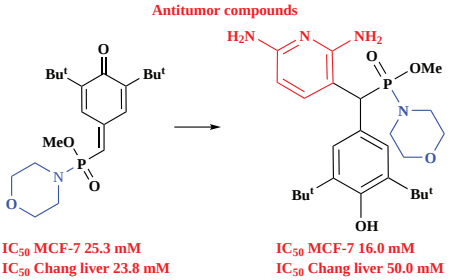
Novel (diarylmethyl)phosphonamidates containing 2,6-di-*tert*-butylphenol and heterocycle moieties were synthesized by 1,6-conjugate addition of phosphorylated *p*-quinone methide with morpholine fragment to 2,6-diaminopyridine or 1,3-diaminobenzene. In the case of an acid-catalyzed reaction of *p*-quinone methide with sesamol, morpholine was cleaved to form 1,2-benzoxaphosphole substituent. Cytotoxic effects of starting compounds and obtained products were evaluated towards human cancer and normal cells.



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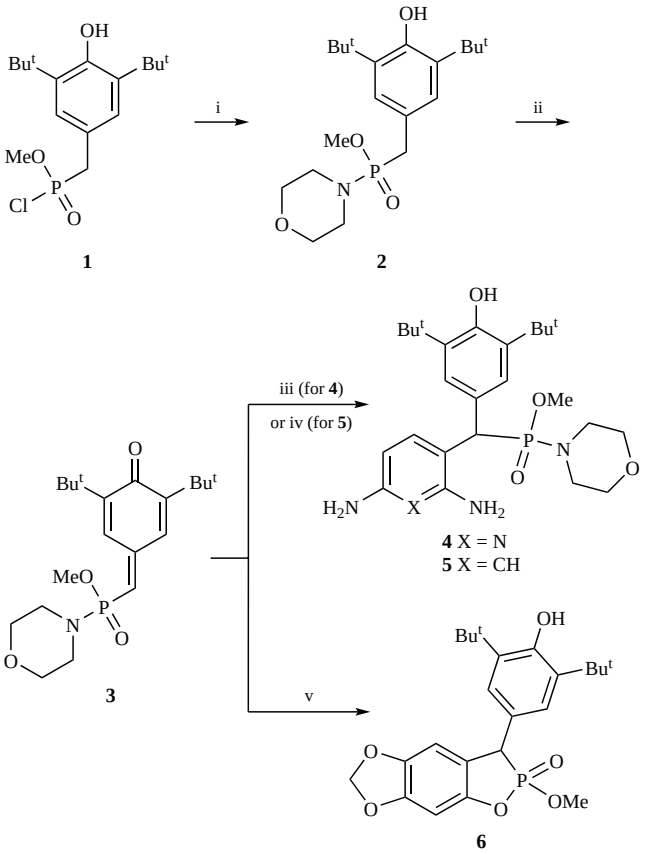
Cancer is an international problem since GLOBOCAN estimated that about 19.3 million new cases of cancer have been registered in 2020.[1] Therefore, the search for new compounds with antitumor activity is an important area of research. In the last few years, *p*-quinone methides have become valuable building blocks for the synthesis of a variety of functionalized molecules.[2–4] 1,6-Conjugate addition of these molecules with a range of carbon- and heteroatom-centered nucleophiles have become important in furnishing diverse scaffolds containing diarylmethane unit.[4–8] Since *p*-quinone methides with 2,6- positioned bulky *tert*-butyl groups are more accessible and more stable, the literature presents works mainly based on them.[2] It should be noted that compounds synthesized on their basis are in turn the derivatives of sterically hindered phenols (SHP) which belong to a family of phenolic antioxidants.[9] Compounds containing SHP moieties are known to exhibit anti-tumor activity.[10–14] The stability of the quinonoid form is strongly influenced by the presence of electron-withdrawing groups in the *para* position.[15,16] In this regard, derivatives of *p*-quinone methides containing a phosphoryl fragment are promising in the synthesis of (diarylmethyl)phosphonates.[17,18] Among organophosphorus compounds, phosphamides and phosphonamidates have recently been used as modifiers for accessing compounds with anticancer potential.[19,20] These compounds can bind to DNA, RNA and some proteins including enzymes.[19–22] The geometry of phosphonamidates, in which the nitrogen atom is bonded to phosphorus(V), is tetrahedral, making the P-stereogenic center that has a key effect on their properties.[23] Meanwhile, heterocyclic motif is frequently encountered in the structures of approved anticancer drugs,[24,25] with morpholine being one of the most common structural fragments.[26,27] The presence of a morpholine fragment at the phosphorus atom can improve the pharmacokinetic properties of the obtained compounds.[27] The high biological activity of morpholine as a scaffold in medicinal chemistry allowed us to choose it as an additional fragment for introduction into SHP molecules.

Previously, we proposed a method for the synthesis of (diarylmethyl)phosphonates exhibiting high antitumor activity, based on the reaction of C-arylation of 2,6-diaminopyridine with dialkyl / diphenyl (3,5-di-*tert*-butyl-4-oxocyclohexa-2,5-dienylidene)methylphosphonates.[18] This method is suitable for the design of molecules containing a diarylmethane stereocenter; in addition, the presence of terminal amino groups opens opportunities for subsequent modification. In this study, we propose to produce *p*-quinone methides containing a phosphonamidate fragment and to determine the possibility of their use for the design of new (diarylmethyl)phosphonamidates. Our approach (Scheme 1) includes initial synthesis of *O*-methyl (3,5-di-*tert*-butyl-4-hydroxybenzyl)phosphonochloridate **1** and its reaction with two equivalents of morpholine in 1,4-dioxane at room temperature to form the corresponding phosphonamidate **2**. The following oxidation of compound **2** with potassium hexacyanoferrate affords novel *p*-quinone methide **3** containing methoxy and morpholine groups at the phosphorus atom. The ^{31}P NMR spectra contain signals at 31.12 ppm for compound **2** and at 17.76 ppm for **3**. The change in chemical shifts of the protons of the methanetriyl group and the SHP fragment in the ^{1}H NMR spectra proves that the oxidation of compound **2** proceeded completely with the formation of *p*-quinone methides **3** (for details, see Online Supplementary Materials).

p-Quinone methide **3** was reacted with 2,6-diaminopyridine or 1,3-diaminobenzene in 1,4-dioxane at room temperature to form 1,6-addition products of mono- and diarylphosphonamidates. Using column chromatography, it was possible to isolate the C-arylation products **4** or **5**, respectively, with ~60%



Scheme 1 *Reagents and conditions*: i, morpholine, 1,4-dioxane, argon; ii, $K_3[Fe(CN)_6]$, 2N KOH, PhH, room temperature; iii, 2,6-diaminopyridine, 1,4-dioxane, room temperature (for **4**); iv, 1,3-$(H_2N)_2C_6H_4$, 1,4-dioxane, room temperature (for **5**); v, sesamol (1,3-benzodioxol-5-ol), CF_3SO_3H, CH_2Cl_2, room temperature.

yields. Both compounds **4**,**5** have two chiral centers at the carbon atom of the methanetriyl group and at the phosphorus atom. Analysis of the ^{1}H, ^{13}C, and ^{31}P NMR spectra showed that they were formed as diastereomer mixtures.

We employed sesamol (1,3-benzodioxol-5-ol) in the reaction with *p*-quinone methide **3** to find out the possibility of forming phosphonamidates containing a diarylmethane scaffold (see Scheme 1). The reaction resulted in the displacement of the morpholine fragment to afford a polycyclic product **6**. ^{31}P NMR spectrum of the reaction mixture contained a signal at 46.65 ppm, which should correspond to 1,2-benzoxaphospholes.[28,29] 2-D Correlation NMR spectroscopy experiments (^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC) truly confirm the structure of **6**. The ^{1}H–^{13}C HMBC spectrum (Figure 1) contained cross peaks between the methanetriyl group proton H^7 at the phosphorus atom and $C^{3,4,8,9,14}$ carbon atoms as well as the H^3 proton and $C^{1,5}$ carbon atoms. This would suggest the formation of a cyclic structure (see also Online Supplementary Materials).

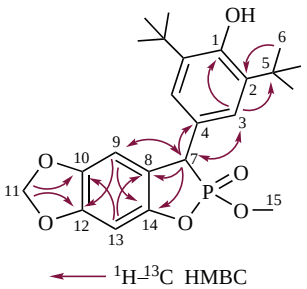
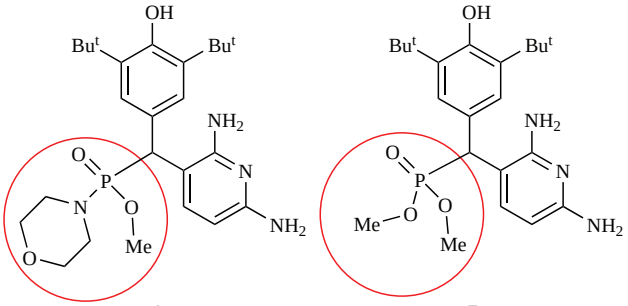


Figure 1 Key cross-peaks in the ^{1}H–^{13}C HMBC spectra of compound **6**.

Table 1 Cytotoxic effects IC_{50} of test compounds on the cancer and normal human cell lines.[a]

Compound	IC_{50} (μM)		
	Cancer cell lines		Normal cell line
	M-HeLa	MCF-7	Chang liver
2	>100	81.0 ± 6.4	>100
3	53.5 ± 4.2	25.3 ± 1.9	23.8 ± 1.8
4	27.2 ± 2.1	16.0 ± 1.2	50.0 ± 3.9
5	76.8 ± 5.9	84.8 ± 6.7	73.9 ± 5.8
7[b]	16.1 ± 1.3	31.0 ± 2.6	>100
Tamoxifen	28.0 ± 2.5	25.0 ± 2.2	46.2 ± 3.5

[a] The experiments were repeated three times. The results are expressed as the mean ± standard deviation. [b] Ref. 18.



Compounds **2**–**5** were tested for cytotoxicity against human normal and cancer cell lines at concentrations of 1–100 μM (Table 1). The effects were estimated with the use of the multifunctional Cytell Cell Imaging system (GE Health Care Life Science, Sweden) using the Cell Viability Bio App which precisely counts the number of cells and evaluates their viability by detecting fluorescence intensity.[30] The starting compound **2** did not show antitumor activity while quinone **3** exhibited cytotoxicity against MCF-7 (the human breast adenocarcinoma) cell line as well as against normal cell line. The 2,6-diaminopyridine derivative **4** containing morpholine and methoxy substituents has *ca.* twofold higher cytotoxicity towards the MCF-7 cancer cell line than its structural analogue **7** with two methoxy substituents[18] and more toxic to the normal Chang liver line. Compound **5** bearing a 1,3-diaminobenzene moiety showed no cytotoxicity.

In summary, compound **4** demonstrated cytotoxicity to MCF-7 (IC_{50} 16.0 μM), which is 1.5-fold higher than the activity of the antitumour drug Tamoxifen. The present study strongly suggests that *p*-quinone methide containing a morpholine fragment is a promising scaffold for the design of potent anticancer drugs.

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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.2023.02.027.

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