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Original article

Design, cytotoxic and fluorescent properties of novel *N*-phosphorylalkyl substituted *E*,*E*-3,5-bis(arylidene)piperid-4-ones

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ABSTRACT

A series of *E*,*E*-*N*-phosphorylalkylene-3,5-bis(arylidene)piperid-4-ones **7a**–**k** was prepared via the condensation of aromatic aldehydes with ω-aminophosphonates **5a**–**c** and **6a**,**b** bearing piperidone or a protected piperidone moiety, respectively. The synthetic routes to the starting aminophosphonates **5a**–**c** and **6a**,**b** varied depending on the number of methylene groups in the alkylene chain and comprised the Kabachnik–Fields reaction ($n = 1$), the aza-Michael reaction ($n = 2$) or alkylation of 4-piperidone hydrochloride with diethyl ω-bromoalkylphosphonates under phase transfer catalysis conditions ($n = 3,4$). Phosphoryl substituted 3,5-bis(arylidene)piperid-4-ones **7b**,**c**,**e**,**f**,**h**,**i**,**k** bearing both nitro groups and fluorine atoms in the *para*-position of the arene rings possess cytotoxicity toward human carcinoma cell lines CaOv3, Scov3, PC3 and A549 in the low micromolar range while their analogues having *para*-dimethylamino groups had IC_{50} values greater than 50 μM . In contrast, only Me_2N-substituted phosphonates **7g**,**j** ($n = 3$ and 4) and the salts of Me_2N-substituted phosphonic acids **10c**,**f** ($n = 2$ and 3) display fluorescence.

1. Introduction

For years γ-piperidone derivatives have been effectively used as a base for design of pharmacologically active compounds such as psycholeptics (e.g. the butyrophenone derivatives), local anesthetics (diammokann – 1-(β-anilinoethyl)-4-phenyl-4-(β-diethylaminoethoxy)piperidine *N*-cyclohexylsulfamine salt), analgetics such as desprodin, prodin, meprodin, promedol, phentranyl and its analogues which are currently used in clinical practice.

Recently, 3,5-bis(arylidene)-4-piperidones **1** (Fig. 1) were found to possess high cytotoxic and antitumor activity towards different cell lines [1–9] while hydrochloride salt of 3,5-bis(benzylidene)-4-piperidone was well tolerated in mice whereby five daily doses up to 240 mg/kg did not cause mortalities [10]. Moreover, these compounds (generally screened as free bases) are non-lethal and mice tolerated doses up to and including 300 mg/kg [11]. 3,5-Bis(arylidene)-4-piperidones display anticancer properties via the supposed mechanism of action comprising interaction with cellular thiols with little or no affinity for hydroxyl and amino groups in nucleic acids [9,12]. Cytotoxic properties were found for the related cycloalkan-1-ones **2** possessing a conjugated structure [13–15] and for curcumin **3** (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione), a natural component of the rhizome of *Curcuma longa*, which proved to be the powerful chemopreventive and anticancer agent [16,17] having also anti-inflammatory [18], antibacterial [19], antihepatoxic [20], and antioxidative [21] properties. Curcumin and other enones are supposed to have multiple targets and interacting macromolecules within the cell [6,16,17]. The 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore group observed both in alicyclic unsaturated enones and cyclic piperidone derivatives is speculated to interact with cellular constituents, while the nature of the group on the heterocyclic nitrogen atom in the piperidone derivatives could influence the cytotoxic properties of the compounds. In other words, it can increase the cytotoxic properties by facilitating approach of the cytotoxin to a specific binding site or reduce them preventing this interaction.

Recent observations demonstrate fluorescent properties of some representatives of 3,5-bis(arylidene)-4-piperidones **1** [22–24].

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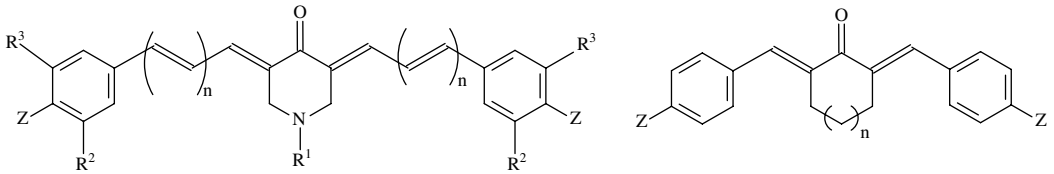
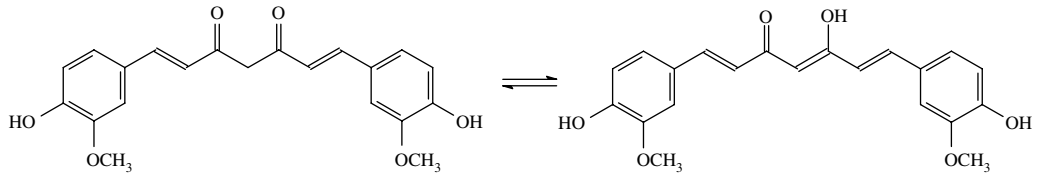


1, R^1 = H, Alk; R^2, R^3=H, OH; n = 0, 1; Z = NMe_2, NO_2, F

2, Z = $C(NH)NH_2$, n = 0-5



3, Curcumin

Fig. 1.

Natural fluorescence in the case of cytotoxic materials might be very helpful in tracking their target organelle in cell. In the case of piperidinones and their derivatives the cytotoxic activity increases with the increase of electron withdrawing properties of the phenyl ring substituents, which at the same time decreases the fluorescent activity. Combination of antitumor activity and fluorescent properties makes these types of compounds exceedingly attractive as drug candidates.

As mentioned above, antitumor activity and fluorescent properties of 3,5-bis(arylidene)piperid-4-ones are predominantly determined by the conjugated system while solubility and bioavailability are largely governed by the nature of the groups at the nitrogen atom of the piperidone moiety. However, the π-values of the aryl substituents can affect both these parameters. These groups may possess inherent bioactivity, and it would be beneficial if they could enhance the therapeutic effect resulting in additional impact on cancer cells due to the different mechanisms of action. So, the modification of the structures of 3,5-bis(arylidene)piperid-4-ones both by variation of substituents at aromatic rings and by insertion of bioactive groups to the piperidone nitrogen atom is a very challenging route to novel fluorescent cytostatics. The attempts to improve the antitumor properties of 3,5-bis(arylidene)-4-piperidones and affect the capacity of their transportation via the cellular membrane were mostly concentrated on modification at the nitrogen atom as *N*-acylation [2,4] or *N*-phosphorylation [22].

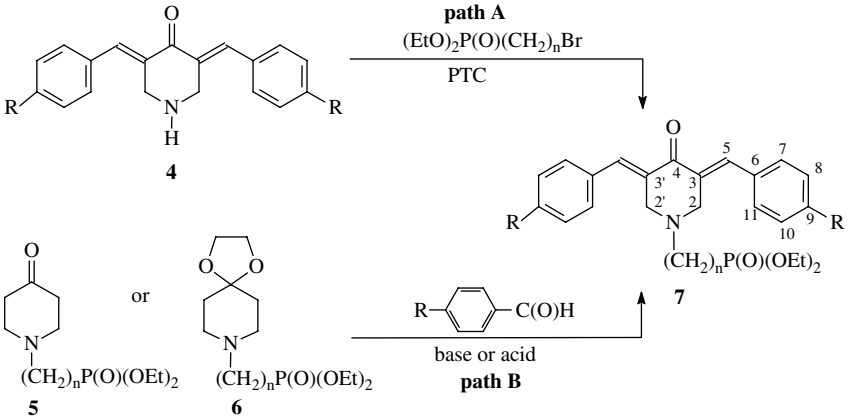
It should be mentioned that organophosphorus compounds exhibit different types of bioactivity, and therefore form an important series of compounds in the search for new drugs. For instance, high-level anticancer activity has been found in a number of phosphorus compounds of quite different structural types, among them discovered in 1958 cyclophosphamide [25], which still remains in wide clinical use, *N*-phosphonoacetyl-L-aspartic acid (PALA) [26], derivatives of choline, including ether derivatives of Lysophosphatidyl choline [27], 6–8 membered 1,2-azaphosphacyclanes [28] etc. Moreover, phosphorylated compounds may be used as prodrugs to improve drug delivery to particular targets and to increase the solubility of drugs.

Taking into account bioactivity of organophosphorus compounds in general and high and diverse biological activity of aminophosphonates or so-called "phosphorus analogues" of aminoacids in particular [29], it seems reasonable to synthesize and evaluate the cytotoxic and fluorescent properties of 3,5-bis(arylidene)piperidones bearing the ω-phosphonate group in the *N*-alkylene chain.

2. Results and discussion

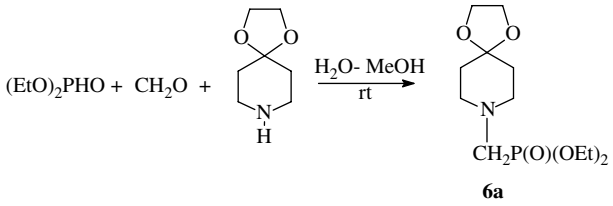
2.1. Chemistry

For the synthesis of the desired *N*-(ω-phosphorylalkyl)-3,5-bis(arylidene)piperid-4-ones two possible synthetic routes can be



R=NMe_2, F, NO_2; n=1-4

Scheme 1. Possible synthetic approaches to *N*-phosphorylalkylated 3,5-bis(arylidene)piperidones **7**.

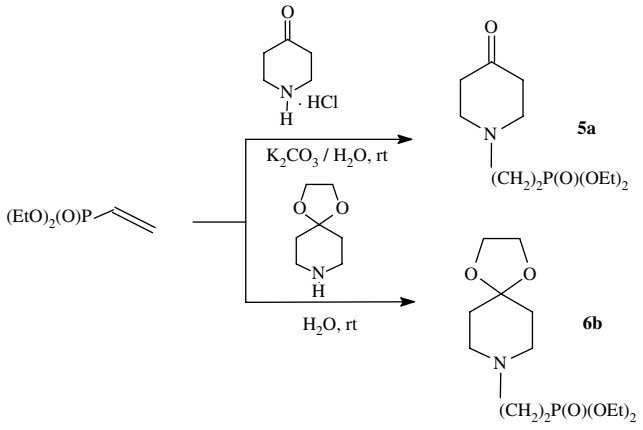


Scheme 2. Synthesis of α-aminomethylphosphonate via the Kabachnik-Fields reaction.

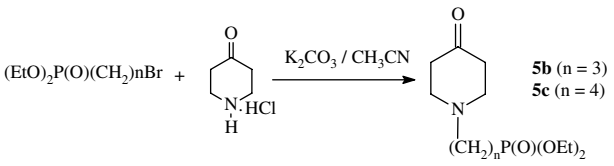
used namely either alkylation of parent NH-3,5-bis(arylidene)piperid-4-ones **4** by phosphorylated halogenoalkanes or condensation of preformed *N*-phosphorylalkyl substituted piperidones **5** or their *O*-protected derivatives **6** with aromatic aldehydes under basic or acid conditions as outlined on Scheme 1. However, previously we demonstrated [30] that reaction of NH-3,5-bis(arylidene)piperid-4-ones **4** with short-chain halogenoalkanes proceeded predominately as quaternization to afford *N*,*N*-dialkylated products. Therefore, to avoid such side reaction condensation of ω-(4-oxopiperidin-1-yl)alkylphosphonates **5** or their protected forms **6** with aromatic aldehydes was the method of choice.

The synthetic routes to the starting aminophosphonates bearing piperidone moiety **5** or their protected derivatives **6** varied depending on the number of methylene groups of the alkylene chain. Thus, *N*-phosphorylmethyl substituted piperidone ($n = 1$) was obtained as the dioxolane protected form **6a** via the three-component Kabachnik–Fields reaction of diethylphosphite, commercially available 1,4-dioxa-8-azaspiro[4.5]decane and formaldehyde in aqueous media (Scheme 2). Under these conditions the reaction proceeds smoothly to give **6a** as the main reaction product (87% according to the ^{31}P spectra of the reaction mixture). However, distillation of crude product is accompanied by partial decomposition reducing the total yield of purified **6a** to 55%.

For the synthesis of β-aminophosphonates **5a**, **6b** bearing the piperidone or protected piperidone moiety, respectively, we used the aza-Michael reaction (Scheme 3). According to our data [31], water as a solvent significantly accelerates the addition of various amines to diethoxyvinylphosphonate to yield β-aminophosphonates without any catalyst compared to other known procedures for this reaction. When 4-piperidone hydrochloride was used as a starting amine β-aminophosphonate **5a** was isolated in 62% yield after purification by chromatography. In the case of the protected piperidone derivative, 1,4-dioxa-8-azaspiro[4.5]decane, no side reactions were observed and modified piperidone **6b** was isolated in 98% yield with >98% purity after a simple liophilization procedure.



Scheme 3. Synthesis of β-aminoethylphosphonates via the aza-Michael reaction.



Scheme 4. Synthesis of γ-aminopropyl- and δ-aminobutylphosphonates under PTC conditions.

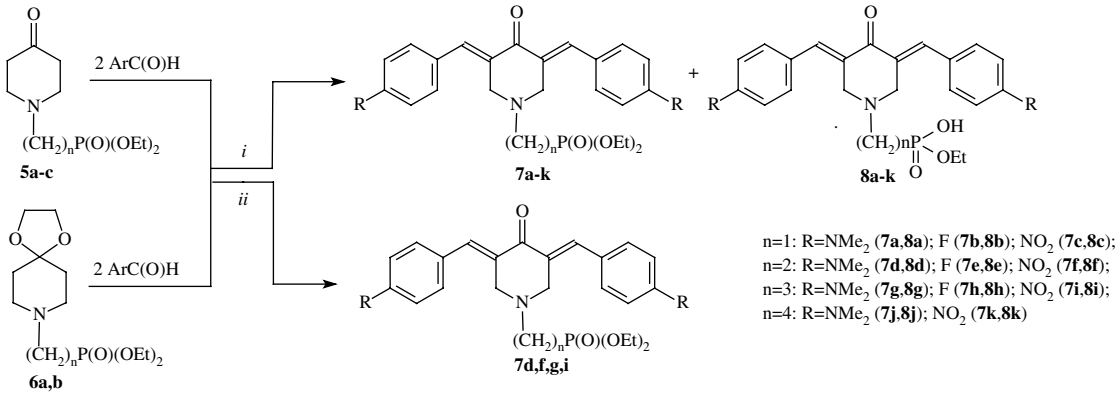
Piperidones **5b**,**c** having three and four methylene groups in the alkylene chain were prepared in high yields by alkylation of 4-piperidone hydrochloride with diethyl ω-bromoalkylphosphonates under phase transfer catalysis conditions (Scheme 4).

As mentioned above, condensation of phosphorylated piperidones **5a**–**c** with substituted benzaldehydes affording the target *N*-phosphorylalkyl substituted 3,5-bis(arylidene)piperidones **7** can be performed both under basic or acidic conditions. In the case of dioxalane protected derivatives **6a**,**b** only acidic conditions were applied in order to ensure hydrolytic removal of the dioxalane protective group (Table 1 and Scheme 5). It should be mentioned that under acidic conditions (bubbling of gaseous HCl into a mixture of reactants in acetic acid) the condensations of compounds **5** and **6** were accompanied by partial dealkylation of one ester group at the phosphorus atom in the phosphonate moiety of the final 3,5-bis(arylidene)piperidones **7a**–**k** (Scheme 5). In some cases the corresponding semiesters **8** were isolated in pure form either as free acids **8b**,**e** (R = F, $n = 1$ and 2, respectively) or as sodium salts **8j**,**g** ($n = 3$, 4, R = NMe_2) after the work-up procedure (see Supplementary information). Such partial hydrolysis reduced the yields of the 3,5-bis(arylidene)piperidones **7** and made work-up and purification procedures difficult, especially in the case of *p*-dimethylaminoarylidene derivatives where stable emulsions of crude products in water were formed. The condensation under basic conditions was the reasonable alternative to the acid-mediated process, however, using alcoholic NaOH lead to a significant decrease in product yields (less than 15%). Better results were obtained when the reaction was performed in the presence of piperidine as a base in alcoholic solutions. Under such conditions the final products **7d**,**f**,**g**,**i** were isolated in the lower yields compared to acid-catalyzed condensation but the isolation procedure was more convenient (see Section 4).

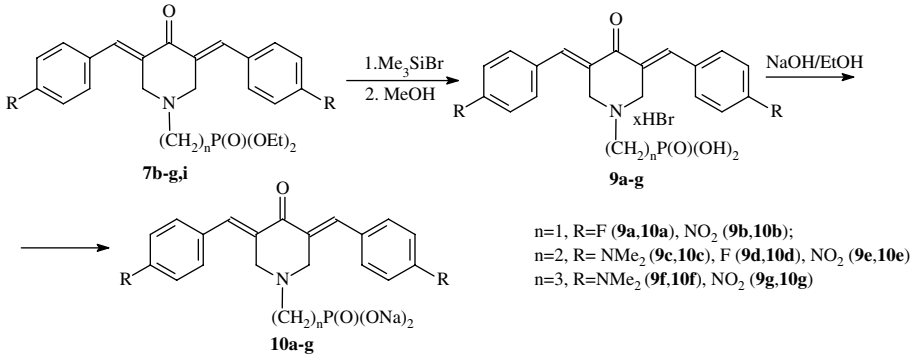
Total hydrolysis of ester groups at the phosphorus atoms in phosphonates **7b**–**g**,**i** was performed via the reaction with trimethylsilylbromide in chloroform followed by the treatment with aq.

Table 1
Synthesis of phosphonates **7a**–**k** via Scheme 5.

Compound	*n*	R	Conditions/starting substrate	Yield, %
7a	1	NMe_2	i/**6a**	34
7b	1	F	i/**6a**	42
7c	1	NO_2	i/**6a**	43
7d	2	NMe_2	i/**5a**	34
7d	2	NMe_2	i/**6b**	37
7d	2	NMe_2	ii/**6b**	34
7e	2	F	i/**5a**	27
7e	2	F	i/**6b**	40
7f	2	NO_2	i/**5a**	40
7f	2	NO_2	i/**6b**	57
7f	2	NO_2	ii/**6b**	29
7g	3	NMe_2	i/**5b**	32
7g	3	NMe_2	ii/**5b**	27
7h	3	F	i/**5b**	37
7i	3	NO_2	i/**5b**	47
7i	3	NO_2	ii/**5b**	23
7j	4	NMe_2	i/**5c**	43
7k	4	NO_2	i/**5c**	30



Scheme 5. Synthesis of phosphonates **7a–k**. Reagents and conditions: (i) HCl(g)/AcOH then K_2CO_3/H_2O; (ii) piperidine/EtOH.



Scheme 6. Hydrolysis of phosphonates **7**.

MeOH. The corresponding phosphonic acids **9a–g** were isolated either as hydrobromides or as hydrobromides-hydrates containing fractional amounts of hydrogen bromide and water molecules per one molecule of acid. For evaluation of antitumor activity, the acid hydrobromides **9a–g** were further converted to water-soluble phosphonic acid sodium salts **10a–g** by treatment with an alcoholic solution of NaOH (Scheme 6).

2.2. Fluorescence

Taking into account the fluorescent properties of some arylidenepiperidones [22–24,30] giving the possibility to estimate their distribution in cancer cells [30], absorption and emission spectra of some phosphonates **7g–k** and salts **10a–g** were studied. Absorption and emission maxima of all compounds are listed in Table 2. It can be seen that compounds **7h** and **7i**,**k** bearing fluorine and nitro groups in arene rings correspondingly have one absorption peak around 330 nm while those **7g**,**j** bearing dimethylamino groups have two absorption peaks, one around 270 nm and the second larger one around 455 nm. The absorption spectra of phosphonates **7g–k** in chloroform are shown in Fig. S1. Similarly, two absorption peaks, one around 280 nm and the second one around 475 nm are observed for absorption spectra of salts **10c**,**f** ($R = NMe_2$) in water (Fig. S2). Moreover, two absorption peaks (higher at 330 nm and lower at 235 nm) were observed for salts **10a**,**d** bearing *p*-F moieties and only one peak for *p*-nitrosubstituted derivatives.

To obtain fluorescent spectra, compounds were excited at $\lambda_{ex} = 400$ nm (in the case of **7g**,**j** and **10c**,**f**). The fluorescence emission was measured at the wavelength interval of 410–800 nm. It was also found that higher intensity emission is observed when solutions are excited near the maximum of a longer wavelength absorption peak. Practically no fluorescence is observed both for phosphonates and phosphonic acid salts having fluorine or nitro-substituents, while compounds **7g**,**j** and **10c**,**f** possess intense fluorescence at ca. 565 nm (Figs. 2 and 3). Fluorescence quantum yields were determined using the fluorescence standard 9,10-diphenylanthracene, 1×10^{-5} M, dissolved in degassed ethanol. Values were determined by the procedure of Maciejewski and Steer [32].

2.3. Cytotoxic properties

The cytotoxic activity of the compounds synthesized was tested against four human cell lines, namely CaOv3 and Scov3 (ovarian carcinoma), PC3 (prostate carcinoma) and A549 (lung carcinoma).

Table 2
Photophysical data for phosphonates **7g–k** and salts **10a–g**.

Compound	Absorption maximum (nm)	Fluorescence maximum (nm)	Estimated fluorescence quantum yield[a]
7g	455	566	0.022
7h	329	417	4.638E-5
7i	331	385	1.962E-5
7j	456	567	0.024
7k	331	391	8.719E-6
10a	331	392	4.102E-5
10b	327	388	3.231E-5
10c	476	570	0.011
10d	331	394	4.565E-5
10e	326	389	2.674E-5
10f	476	570	0.011
10g	327	382	5.506E-5

[a] Relative to the fluorescence standard 9,10-diphenylanthracene, 1×10^{-5} M, in ethanol.

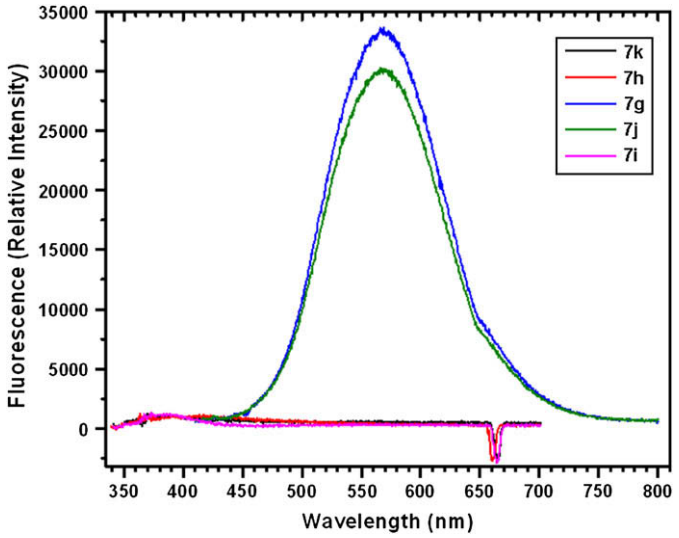


Fig. 2. The fluorescence spectra of phosphonates **7g–k** in chloroform.

The results are summarized in Table 3 showing the corresponding IC_{50} values (IC_{50} is the concentration of compound required to inhibit the growth of the cells by 50%). Anticancer agent melphalan (sarcolysin) was used as a positive control similar to assays of cytotoxic properties of other 3,5-bis(arylidene)-4-piperidone derivatives described in literature [1–9,22,30]. For comparison the activity of parent NH-piperidones **4a–c** bearing the same substituents in the phenyl rings was estimated in the same assay.

The parent NH-piperidones **4a–c** and their phosphorylated analogues **7** excluding those bearing NMe_2 substituents (namely, **4a** for CaOv3 cell line, **7a** for CaOv3, PC3 and A549 screens and 7**d**,**g**,**j** for all cell lines used in these assays) had lower IC_{50} figures than melphalan. In general, the cytotoxic properties of both the NH-piperidones **4a–c** and the *E*,*E*-*N*-phosphorylalkylene-3,5-bis(arylidene)piperid-4-ones **7a–k** fit well with the above mentioned general tendency that cytotoxic activity increases with the increase of electron withdrawing properties of the substituents R in the *para*-position of benzene rings. Thus, compounds **7c**,**f**,**i**,**k** ($n = 1, 2, 3$ and 4 respectively) having strong electron withdrawing nitro group in the *para*-position of benzene rings are the most active in this series. The presence of the both weak acceptor and weak electron-donating

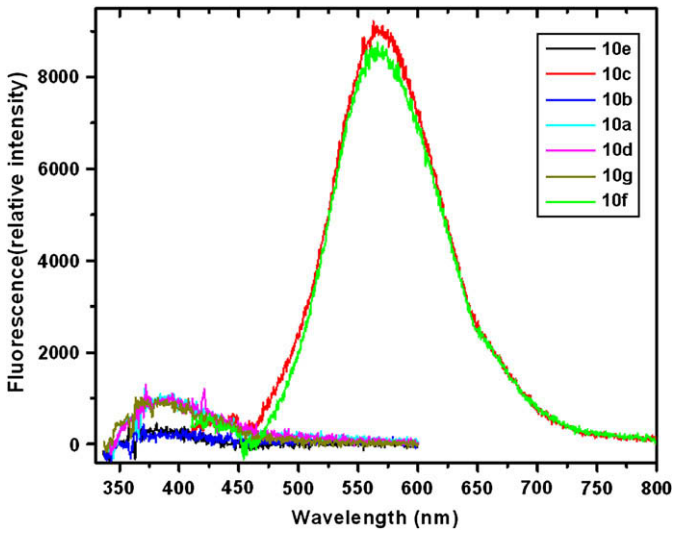


Fig. 3. The fluorescence spectra of phosphonic acid sodium salts **10a–g** in water.

Table 3
Cytotoxicity of ω-aminophosphonates **7a–k** and parent NH-piperidones **4a–c** against human CaOv3, Scov3, PC3 and A549 cell lines.

Compound	R	*n*	CaOv3	Scov3	PC3	A549
			IC_{50} (μM)[a]	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
4a	NMe_2	–	NT[a]	14 ± 4.5	15 ± 5	1.4 ± 0.4
4b	F	–	NT[a]	6.5 ± 1.5	6 ± 3	0.80 ± 0.07
4c	NO_2	–	1.03 ± 0.09	2.5 ± 1.0	1.54 ± 0.35	0.4 ± 0.1
7a	NMe_2	1	NT[a]	NT[a]	30 ± 3.21	NT[a]
7b	F	1	10.20 ± 0.78	2.1 ± 0.31	3.0 ± 0.6	8.05 ± 0.09
7c	NO_2	1	5.03 ± 0.57	1.52 ± 0.23	1.40 ± 0.23	1.0 ± 0.2
7d	NMe_2	2	NT[a]	NT[a]	NT[a]	NT[a]
7e	F	2	10.04 ± 1.12	1.64 ± 0.15	4.01 ± 0.23	1.02 ± 0.13
7f	NO_2	2	5.05 ± 0.68	2.01 ± 0.22	1.32 ± 0.21	1.00 ± 0.05
7g	NMe_2	3	NT[a]	NT[a]	NT[a]	NT[a]
7h	F	3	8.50 ± 0.03	2.8 ± 0.3	1.82 ± 0.44	9.07 ± 0.32
7i	NO_2	3	5.62 ± 0.21	0.9 ± 0.3	0.71 ± 0.08	7.12 ± 0.41
7j	NMe_2	4	NT[a]	NT[a]	NT[a]	NT[a]
7k	NO_2	4	1.01 ± 0.07	4.1 ± 0.2	1.2 ± 0.2	3.0 ± 0.3
Melphalan	–	–	20 ± 5	60 ± 10	70 ± 20	50 ± 20

[a] NT means that at concentration 50 μM 50% cell death was not observed, further increase of the concentration usually resulted in precipitation of the compound.

fluorine atom (compounds **4b** and **7b**,**e**,**h**) generally decreases the activity in comparison with their analogues with a nitro-substituent and the same length of the linker (compounds **7**). It should be noted that dimethylamino substituted *N*-ω-phosphorylated compounds **7a**,**d**,**g**,**j** do not demonstrate cytotoxicity, while compound **4a** (lines Scov3, PC3 and A549) and non-phosphorylated *N*-alkyl-3,5-bis(arylidene)piperid-4-ones and their quaternized salts possess cytotoxic activity even when they have *para*-NMe_2 or NEt_2 groups in benzene rings [line A549, see Ref. [30]].

For compounds **7b**,**e**,**h** bearing the *para*-F atom, the introduction of phosphorylalkyl substituent resulted in the increase of cytotoxic properties for CaOv3, Scov3, and PC3 cells in comparison with the NH-piperidone **4b** while the tendency changed for the opposite one for A549 screen. Such clear tendency was not observed in the case of phosphonates having the *para*-NO_2 groups possessing either higher or lower activities in comparison with those for **4c** depending on the alkylene chain length between the phosphorus atom and the heterocyclic nitrogen one. Thus, for Scov3 phosphonates **7c**,**f**,**i** are more active than **4c**, for A549 the cytotoxic properties of **4c** are higher than those of its analogues **7c**,**f**,**i**,**k**, for CaOv3 cell line only activity of δ-aminophosphonate **7k** was similar to those of **4c** while for PC3 IC_{50} values of all phosphorylated compounds **7c**,**f**,**i**,**k** were less comparing with **4c**.

Moreover, the influence of the alkylene chain length manifested variously for different cell lines. Thus, other conditions being equal, δ-aminophosphonate **7k** demonstrates higher activity against the CaOv3 cell line than its analogues with a shorter linker and was just as potent as **4c**. For Scov3 the tendency is in the opposite direction and the activity of **7i** ($n = 3$) was even higher than that for **4c**. For prostate carcinoma PC3 the activity is a little bit lesser than that for **4c**, comparable for **7c**, **7f** ($n = 1$ and 2 correspondingly), and a little bit decreased with elongation of the alkylene chain (compounds **7h**,**k**). At the same time for lung carcinoma cell line A549, β-aminophosphonates **7c**,**e**, and **f** are the most active.

Pharmaceutically acceptable salts are, because their solubility in water is greater than that of the initial compounds, particularly suitable for medical applications. Surprisingly, we failed to obtain 50% inhibition of the cells growth using salts **10** even those having nitro-substituents.

3. Conclusion

We have developed efficient synthetic approaches to ω-aminophosphonates bearing piperidone or a protected piperidone

moiety and differing in alkylene chain length. These are useful as starting substrates for the synthesis of *E,E-N*-phosphorylalkylene-3, 5-bis(arylidene)piperid-4-ones. Investigation of cytotoxic properties of the compounds has revealed that a number of phosphonates in the 3,5-bis(arylidene)piperid-4-one series have IC_{50} values in the low micromolecular range towards four human carcinoma cell lines. The difference in potencies between the compounds is attributed to the difference in electronic properties of substituents R in the *para*-position of the benzene rings rather than alkylene chain length. Namely, the cytotoxicity increases with the increase of electron withdrawing properties of the substituents R. Donor amino groups result in increased fluorescence emission. This study has revealed the high potential of these molecules for further development to improve potencies.

4. Experimental protocols

4.1. General remarks

NMR spectra were recorded with a Bruker AMX-400 spectrometer (^{1}H, 400.13; ^{19}F, 376.3; ^{31}P, 161.97 and ^{13}C, 100.61 MHz) using residual proton signals of deuterated solvent as an internal standard (^{1}H, ^{13}C), $CFCl_3$ (^{19}F) and H_3PO_4 (^{31}P) as an external standard. The ^{13}C NMR spectra were registered using the JMODECHO mode; the signals for the C atom bearing odd and even numbers of protons have opposite polarities. Melting points are uncorrected. HRMS were obtained on a Varian MAT CH7A instrument at 70 eV. Analytical TLCs were performed with Merck silica gel 60 F_{254} plates. Visualization was accomplished by UV light. IR spectra were recorded in KBr pellets on a Fourier-spectrometer "Magna-IR750" (Nicolet), resolution 2 cm^{-1}, 128 scans. The typical intensive absorption bands in the 1200–1250 cm^{-1} regions which may be unambiguously assigned to vibrations of the P=O moiety were not observed in the IR spectra of the phosphonates **7** as they are mixed with vibrations of the conjugated aromatic system.

The starting diethoxy(3-bromopropyl)phosphonate [33] and diethoxy(4-bromobutyl)phosphonate [34] were obtained by the Arbuzov reaction of triethylphosphite and 1,3-dibromopropane or 1,4-dibromobutane correspondingly according to the known procedures. Other reactants were purchased from Aldrich and used without further purification.

4.2. Diethyl 2-(4-oxopiperidin-1-yl)ethylphosphonate ***5a***

To a solution of 4-piperidone hydrochloride monohydrate (4.62 g; 0.03 mol) in deionised water (15 mL) at 0 °C a solution of potassium carbonate (4.14 g, 0.03 mol) in water (10 mL) was added. Then diethyl vinylphosphonate (4.92 g, 0.03 mol) was added, and reaction mixture was stirred at room temperature for two days. The reaction solution was extracted with CH_2Cl_2, the organic extract was dried over Na_2SO_4, filtered and evaporated at reduced pressure to give the crude product (6.27 g, 78%) which was purified by column chromatography on SiO_2 (gradient from 100% $CHCl_3$ to $CHCl_3$/EtOH = 20:1) to give 4.9 g (62%) of the title compound. ^{1}H ($CDCl_3$), δ: 1.22 (t, 6H, $OCH_2\underline{CH_3}$, $^3J_{HH} = 7$ Hz), 1.89 (dt, 2H, $CH_2\underline{CH_2}P$, $^2J_{PH} = 20$ Hz, $^3J_{HH} = 8$ Hz), 2.34 (appeared t, 4H, $N(CH_2\underline{CH_2})_2$ cyclic, $^3J_{HH} = 6$ Hz), 2.66 (m, 6H, $N(CH_2\underline{CH_2})_2$ cyclic and $\underline{CH_2}CH_2P$), 4.00 (m, 4H, $O\underline{CH_2}CH_3$). ^{31}P ($CDCl_3$), δ: 31.24. Anal. calcd. for $C_{11}H_{22}NO_4P$: C 50.18, H 8.42, N 5.32, P 11.76%; found: C 49.93, H 8.58, N 5.18, P 11.44%.

4.3. Diethyl 3-(4-oxopiperidin-1-yl)propylphosphonate ***5b***

A mixture of diethyl 3-bromopropylphosphonate (5.0 g, 0.0193 mol), piperidone monohydrate hydrochloride (2.97 g, 0.0193 mol) and potassium carbonate (6.5 g, 0.047 mol) in CH_3CN (40 mL) was stirred for ten days at room temperature until completion of the reaction (monitoring via ^{31}P NMR spectra). Insoluble substances were filtered off and washed with CH_2Cl_2 (20 ml). Organic solutions were combined, evaporated in vacuum to dryness and the residue was extracted by boiling hexane (4 × 25 ml). Hexane was removed in vacuum to give diethyl 3-(4-oxopiperidin-1-yl)propylphosphonate (4.5 g, 84%, purity 94% according to the ^{31}P and ^{1}H NMR spectra) as light-yellow liquid. The compound was used in the next step without further purification. ^{1}H ($CDCl_3$), δ: 1.22 (t, 6H, $POCH_2\underline{CH_3}$, $^3J_{HH} = 7$ Hz), 1.72 (m, 4H, $NCH_2\underline{CH_2}CH_2P$ and $NCH_2CH_2\underline{CH_2}P$), 2.34 (appeared t, 4H, cyclic $N(CH_2\underline{CH_2})_2$, $^3J_{HH} = 5.4$ Hz), 2.42 (t, 2H, $NCH_2CH_2CH_2P$, $^3J_{HH} = 6$ Hz), 2.64 (appeared t, 4H, cyclic $N(CH_2)_2$, $^3J_{HH} = 5.4$ Hz), 4.00 (m, 4H, $POCH_2$). ^{13}C ($CDCl_3$), δ: 15.93 ($POCH_2\underline{CH_3}$, $^3J_{CP} = 6.4$ Hz), 19.89 ($NCH_2\underline{CH_2}CH_2P$, $^2J_{CP} = 4.8$ Hz), 22.69 ($NCH_2CH_2\underline{CH_2}P$, $J_{CP} = 142.5$ Hz), 40.59 (C–C(O)), 52.35 (cyclic $N(CH_2)_2$), 54.64 ($NCH_2CH_2CH_2P$, $^3J_{CP} = 16.8$ Hz), 60.88 ($PO\underline{CH_2}CH_3$, $^2J_{CP} = 6.4$ Hz), 208.19 (CO). ^{31}P ($CDCl_3$), δ: 32.08. Anal. calcd. for $C_{12}H_{24}NO_4P$ (%): C 51.98, H 8.72, P 11.17, found (%): C 51.48, H 8.75, P 11.26.

4.4. Diethyl 3-(4-oxopiperidin-1-yl)butylphosphonate ***5c***

The compound was obtained according the above procedure except that diethyl 4-bromobutylphosphonate was used instead of diethyl 3-bromopropylphosphonate and the reaction time was ca.14 days. Yield 82%, purity 95%. The compound was used in the next step without further purification. ^{1}H ($CDCl_3$), δ: 1.22 (t, 6H, $POCH_2\underline{CH_3}$, $^3J_{HH} = 7.1$ Hz), 1.41–1.61 (m, 6H, $NCH_2\underline{CH_2}\underline{CH_2}\underline{CH_2}P$), 2.34 (appeared quintet, 6H, cyclic $N(CH_2\underline{CH_2})$, $^3J_{HH} = 6$ Hz and $NCH_2CH_2CH_2CH_2P$, $^3J_{HH} = 6$ Hz), 2.68 (appeared t, 4H, cyclic $N(CH_2)_2$, $^3J_{HH} = 6.1$ Hz), 3.88 (m, 4H, $POCH_2CH_3$). ^{13}C ($CDCl_3$), δ: 15.99 ($POCH_2\underline{CH_3}$, $^3J_{CP} = 5.9$ Hz), 19.91 ($NCH_2CH_2\underline{CH_2}CH_2P$, $^2J_{CP} = 5.1$ Hz), 24.95 ($NCH_2CH_2CH_2\underline{CH_2}P$, $J_{CP} = 140.5$ Hz), 27.65 ($NCH_2\underline{CH_2}CH_2CH_2P$, $^3J_{CP} = 16$ Hz), 40.70 ($-H_2\underline{C}-C(O)$), 52.58 ($N(CH_2)_2$ cyclic), 56.19 ($NCH_2CH_2CH_2CH_2P$), 60.91 ($PO\underline{CH_2}CH_3$, $^2J_{CP} = 6.6$ Hz), 208.53 (CO). ^{31}P ($CDCl_3$), δ: 32.00. Anal. calcd. for $C_{13}H_{26}NO_4P \cdot 1/3H_2O$ (%): C 52.51, H 9.04, P 10.42; found (%): C 52.56, H 9.12, P 10.17.

4.5. Diethyl (1,4-dioxa-8-azaspiro[4.5]dec-8-yl)methyl phosphonate ***6a***

1,4-Dioxa-8-azaspiro[4.5]decane (4.29 g, 0.03 mol) was added portionwise to a solution of diethylphosphite (4.14 g, 0.03 mol) and formalin (2.43 g of 37% solution in water–MeOH, 0.03 mol) in deionised water (15 mL) at room temperature, followed by stirring of the reaction mixture over three days at the same temperature. Water was removed under vacuum and the residue was distilled at reduced pressure to give 4.90 g (55%) of phosphonate **6a** of 94% purity. B.p. 140–150 °C (0.3 Hg). Analytically pure sample was obtained by column chromatography on silica gel (gradient elution from EtOAc/cyclohexane = 4:1 to 100% EtOAc). ^{1}H ($CDCl_3$), δ: 1.11 (t, 6H, $OCH_2\underline{CH_3}$, $^3J_{HH} = 7.0$ Hz), 1.51 (appeared t, 4H, cyclic $N(CH_2\underline{CH_2})_2$, $^3J_{HH} = 5.6$ Hz), 2.66 (appeared t, 4H, cyclic $N(\underline{CH_2}CH_2)_2$, $^3J_{HH} = 5.6$ Hz), 2.73 (d, 2H, $\underline{CH_2}P$, $^2J_{PH} = 11.6$ Hz), 3.87 (s, 4H, $O\underline{CH_2}$-$\underline{CH_2}O$), 4.08 (m, 4H, $O\underline{CH_2}CH_3$). ^{13}C ($CDCl_3$), δ: 15.95 (d, $POCH_2\underline{CH_3}$, $^3J_{CP} = 5.6$ Hz), 34.26 (cyclic $NCH_2\underline{CH_2}$), 52.69 (d, cyclic $N\underline{CH_2}$, $^3J_{CP} = 10.4$ Hz), 53.00 (d, NCH_2, $J_{CP} = 163$ Hz), 61.55 (d, $PO\underline{CH_2}CH_3$, $^2J_{CP} = 6.4$ Hz), 63.63 (s, OCH_2CH_2O), 105.98 (O–C–O). ^{31}P ($CDCl_3$), δ: 25.77. HRMS calculated for $C_{12}H_{24}NO_5P$ (M^+) 293.13921, found 293.13962.

4.6. Diethyl [2-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl) ethyl]phosphonate ***6b***

Diethyl vinylphosphonate (8.20 g, 0.05 mol) was added to a stirred solution of protected piperidone (7.15 g, 0.05 mol) in water

(15 mL) at room temperature and the mixture was stirred at the same temperature for ca 20 h. Water was evaporated under reduced pressure to give 15.1 g (98%) of crude **6b** (>98% purity) as a viscous liquid. The product was used for the further reactions without purification. ^{1}H ($CDCl_3$), δ: 1.15 (t, 6H, OCH_2CH_3, $^3J_{HH} = 7.0$ Hz), 1.57 (appeared t, 4H, cyclic $N(CH_2CH_2)_2$, $^3J_{HH} = 5.6$ Hz), 1.80 (dt, 2H, CH_2CH_2P, $^2J_{PH} = 19$ Hz, $^3J_{HH} = 8.2$ Hz), 2.37 (appeared t, 4H, cyclic $N(CH_2CH_2)_2$, $^3J_{HH} = 5.6$ Hz), 2.52 (m, 2H, CH_2CH_2P), 3.78 (s, 4H, OCH_2CH_2O),3.93 (m, 4H, OCH_2CH_3). ^{13}C ($CDCl_3$), δ: 16.05 (d, $POCH_2CH_3$, $^3J_{CP} = 6.0$ Hz), 23.44 (d, NCH_2CH_2P, $J_{CP} = 138$ Hz), 34.32 (s, cyclic NCH_2CH_2), 50.35 (cyclic NCH_2), 50.73 (NCH_2CH_2P), 61.12 (d, $POCH_2CH_3$, $^2J_{CP} = 6.4$ Hz), 63.79 (s, OCH_2CH_2O), 106.53 (O–C–O). ^{31}P ($CDCl_3$), δ: 31.85. HRMS calculated for $C_{13}H_{26}NO_5P$ (M^+) 307.15486, found 307.15540.

4.7. N-(ω-(Diethoxyphosphorylalkyl)-3,5-bis(arylidene)piperid-4-ones) **7** (general procedure)

Method A: dry hydrochloric acid was bubbled through a solution or suspension of the corresponding aminophosphonate **5a–c**, **6a**,**b** (0.01 mol) and aldehyde (0.02 mol) in glacial acetic acid (ca. 20 mL; in case of well soluble 4-fluorobenzaldehyde, volume of AcOH was reduced to 15 mL) over 2–4 h. The reaction mixture was kept at room temperature for 4–5 days. The volatiles were removed under reduced pressure. Diethyl ether (30 ml) was added to the residue to remove unreacted 4-fluoro- or 4-nitrobenzaldehyde and the precipitate formed was filtered off. To this solid CH_2Cl_2 and an aqueous solution of potassium carbonate or sodium carbonate (pH ~ 8–9) were added. After stirring over 10 min the organic phase was separated, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography on SiO_2 (for details of purification see below) followed by crystallization (slow diffusion method) from a mixture of acetone and CH_2Cl_2, or $CHCl_3$ and pentane to give substituted phosphonates **7**.

Method B: a mixture of the corresponding 2-(4-oxopiperidin-1-yl)alkylphosphonate **5a**,**b** or its *O*-protected form **6b**, (0.01 mol), aldehyde (0.02 mol) and piperidine (1 g, 0.012 mol) in EtOH (15 mL) were refluxed for 4 days. Volatiles were evaporated at reduced pressure and the residue was purified by column chromatography followed by additional crystallization from CH_2Cl_2/pentane mixture.

4.7.1. Diethyl 3,5-bis[4-(dimethylamino)benzylidene]-4-oxopiperidin-1-yl-methylphosphonate **7a**

Eluent – EtOAc, crystallization from $CHCl_3$/pentane mixture. Orange-red crystal solid, m.p. 162–164 °C. IR: 1659 (C=O), 1591, 1556, 1523, 1448, 1366, 1298, 1274, 1254, 1171, 1054 (P–O–C), 1022 (P–O–C), 995, 967, 943, 815, 772, 518. ^{1}H (DMSO-d_6), δ: 1.13 (t, 6H, OCH_2CH_3, $^3J_{HH} = 7.0$ Hz), 2.97 (s, 12H, NMe_2), 3.08 (d, 2H, CH_2P, $^2J_{PH} = 12.0$ Hz), 3.87–4.02 (m, 8H, cyclic $NCH_2 + OCH_2CH_3$), 6.73–6.77 (d, 4H, aromatic), 7.31–7.36 (d, 4H, aromatic), 7.49 (s, 2H, CH=). ^{13}C ($CDCl_3$), δ: 16.16 (d, $POCH_2CH_3$, $^3J_{CP} = 6.0$ Hz), 39.88 (NMe_2), 51.89 (d, CH_2P, $^1J_{CP} = 163$ Hz), 56.14 (d, cyclic $N(CH_2)_2$, $^3J_{CP} = 9.4$ Hz), 61.93 (d, $POCH_2CH_3$, $^2J_{CP} = 6.6$ Hz), 111.49 (C(8), C(10), C(8′), C(10′)), 122.97 (C(6) and C(6′)), 128.33 (C(3) and C(3′)), 132.40 (C(7), C(11), C(7′), C(11′)), 137.15 (C(5) and C(5′)), 150.43 (C(9) and C(9′)), 186.39 (C(O)). ^{31}P (DMSO-d_6), δ: 25.28. HRMS calculated for $C_{24}H_{26}N_3O_8P$ (M^+) 511.26000, found 511.26020.

4.7.2. Diethyl 3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl-methylphosphonate **7b**

Eluent – EtOAc, crystallization from CH_2Cl_2/pentane mixture at −10 °C. Light-yellow crystal solid, m.p. 105–110 °C (no sharp melting). IR: 1675 (C=O), 1611, 1598, 1585, 1508, 1294, 1272, 1222, 1186, 1162, 1047 (P–O–C), 1025 (P–O–C), 996, 959, 934, 828, 784, 528, 497. ^{1}H ($CDCl_3$), δ: 1.17 (t, 6H, OCH_2CH_3, $^3J_{HH} = 7.0$ Hz), 2.95 (d, 2H, CH_2P, $^2J_{PH} = 11.6$ Hz), 3.94–4.08 (m, 8H, cyclic $NCH_2 + OCH_2CH_3$), 7.09 (t, 4H, $^3J_{HH} = 9.0$ Hz, $^3J_{FH} = 9.0$ Hz), 7.36 (dd, 4H, $^3J_{HH} = 8.0$ Hz, $^4J_{FH} = 5.0$ Hz), 7.76 (s, 2H, CH=). ^{13}C ($CDCl_3$), δ: 15.91 (d, $POCH_2CH_3$, $^3J_{CP} = 5.9$ Hz), 51.78 (d, NCH_2P, $J_{CP} = 162$ Hz), 55.48 (d, $N(CH_2)_2$ cyclic, $^2J_{CP} = 9.5$ Hz), 61.73 (d, $POCH_2CH_3$, $^2J_{CP} = 7.0$ Hz), 115.37 (d, C(8), C(10), C(8′), C(10′), $^2J_{CF} = 22.0$ Hz), 130.74 (d, C(6) and C(6′), $^4J_{CF} = 3.3$ Hz), 131.87 (C(3) and C(3′)), 132.01 (d, C(7), C(11), C(7′), C(11′), $^3J_{CF} = 8.4$ Hz), 135.44 (C(5) and C(5′)), 162.54 (d, C(9) and C(9′), $J_{CF} = 251$ Hz), 185.98 (C(4)). ^{19}F ($CDCl_3$), δ: −110.99. ^{31}P ($CDCl_3$), δ: 24.72. HRMS calculated for $C_{24}H_{26}F_2NO_4P$ (M^+) 461.15675, found 461.15624.

4.7.3. Diethyl 3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl-methylphosphonate **7c**

Gradient elution starting from CH_2Cl_2 to CH_2Cl_2/EtOH = 100:3; crystallization from CH_2Cl_2/pentane mixture. Light-yellow crystal solid, m.p. 197–200 °C. IR: 1679 (C=O), 1620, 1592, 1511, 1346, 1315, 1304, 1265, 1248, 1197, 1108, 1047 and 1023 (P–O–C), 997, 972, 927, 856, 758. ^{1}H ($CDCl_3$), δ: 1.22 (t, 6H, OCH_2CH_3, $^3J_{HH} = 7.0$ Hz), 3.00 (d, 2H, CH_2P, $^2J_{PH} = 11.6$ Hz), 3.98–4.11 (m, 8H, cyclic $NCH_2 + OCH_2CH_3$), 7.54–7.58 (d, 4H, aromatic), 7.83 (s, 2H, CH=), 8.27–8.32 (d, 4H, aromatic). ^{13}C ($CDCl_3$), δ: 16.24 (d, $POCH_2CH_3$, $^3J_{CP} = 6.0$ Hz), 52.16 (NCH_2P, $^3J_{CP} = 160$ Hz), 55.59 (d, $N(CH_2)_2$ cyclic, $^2J_{CP} = 9.5$ Hz), 62.13 (d, $POCH_2CH_3$, $^2J_{CP} = 6.8$ Hz), 123.71 (C(8), C(10), C(8′), C(10′)), 130.74 (C(7), C(11), C(7′), C(11′)), 134.49 (C(5) and C(5′)), 135.14 (C(3) and C(3′)), 140.92 (C(6) and C(6′)), 147.47 (C(9) and C(9′), 185.76 (C(O)). ^{31}P ($CDCl_3$), δ: 24.08. HRMS calculated for $C_{24}H_{26}N_3O_8P$ (M^+) 515.14575, found 515.14390.

4.7.4. Diethyl 2-[3,5-bis(4-(dimethylamino)benzylidene)-4-oxopiperidin-1-yl]ethylphosphonate **7d**

Eluent EtOAc; crystallization from CH_2Cl_2/pentane mixture. Orange-red crystal solid, m.p. 124–126 °C. ^{1}H ($CDCl_3$), δ: 1.27 (t, 6H, OCH_2CH_3, $^3J_{HH} = 7.0$ Hz), 1.97 (dt, 2H, CH_2CH_2P, $^2J_{PH} = 18.0$ Hz, $^3J_{HH} = 8.0$ Hz), 2.87 (m, 2H, CH_2CH_2P), 3.01 (s, 12H, NMe_2), 3.84 (s, 4H, NCH_2 cyclic), 4.04 (m, 4H, OCH_2CH_3), 6.68–6.72 (d, 4H, aromatic), 7.29–7.34 (d, 4H, aromatic), 7.75 (s, 2H, CH=). ^{13}C ($CDCl_3$), δ: 16.20 (d, $POCH_2CH_3$, $^3J_{CP} = 6.0$ Hz), 24.15 (d, NCH_2CH_2P, $J_{CP} = 138$ Hz), 39.86 (NMe_2), 50.44 (NCH_2CH_2P), 54.49 ($N(CH_2)_2$ cyclic), 61.46 (d, $POCH_2CH_3$, $^2J_{CP} = 6.4$ Hz), 111.45 (C(8), C(10), C(8′), C(10′)), 122.89 (C(6) and C(6′)), 128.47 (C(3) and C(3′)), 132.33 (C(7), C(11), C(7′), C(11′)), 136.74 (C(5) and C(5′)), 150.37 (C(9) and C(9′), 186.36 (C(O)). ^{31}P ($CDCl_3$), δ: 31.49. HRMS calculated for $C_{29}H_{40}N_3O_4P$ (M^+) 525.27565, found 525.27664.

4.7.5. Diethyl 2-[3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl]ethylphosphonate **7e**

Eluent EtOAc; crystallization from CH_2Cl_2/pentane mixture. Light-yellow crystal solid, m.p. 57–60 (first melting) and 90–93 °C (second melting). ^{1}H ($CDCl_3$), δ: 1.26 (t, 6H, OCH_2CH_3, $^3J_{HH} = 7.0$ Hz), 1.90 (dt, 2H, CH_2CH_2P, $^2J_{PH} = 18.0$ Hz, $^3J_{HH} = 8.0$ Hz), 2.84 (m, 2H, CH_2CH_2P), 3.79 (s, 4H, NCH_2 cyclic), 4.03 (m, 4H, OCH_2CH_3), 7.11 (t, 4H, $^3J_{HH} = 8.0$ Hz, $^3J_{FH} = 8.0$ Hz), 7.36 (dd, 4H, $^3J_{HH} = 8.0$ Hz, $^4J_{FH} = 6.0$ Hz), 7.76 (s, 2H, CH=). ^{13}C ($CDCl_3$), δ: 16.20 (d, $POCH_2CH_3$, $^3J_{CP} = 6.0$ Hz), 24.12 (d, NCH_2CH_2P, $J_{CP} = 139$ Hz), 50.47 (NCH_2CH_2P), 54.48 ($N(CH_2)_2$ cyclic), 61.57 (d, $POCH_2CH_3$, $^2J_{CP} = 6.1$ Hz), 115.67 (d, C(8), C(10), C(8′), C(10′), $^2J_{CF} = 21.6$ Hz), 130.93 (d, C(6) and C(6′), $^4J_{CF} = 3.3$ Hz), 132.12 (C(3) and C(3′)), 132.20 (C(7), C(11), C(7′), C(11′)), 135.48 (C(5) and C(5′)), 162.79 (d, C(9) and C(9′), $J_{CF} = 250$ Hz), 186.51 (C(4)). ^{19}F ($CDCl_3$), δ: −110.97. ^{31}P ($CDCl_3$), δ: 30.95. HRMS calculated for $C_{25}H_{28}F_2NO_4P$ (M^+) 475.17240, found 475.17241.

4.7.6. Diethyl 2-[3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl]ethylphosphonate **7f**

Eluent EtOAc; crystallization from acetone/hexane mixture. Yellow crystal solid, m.p. 154–157 °C. ^{1}H ($CDCl_3$), δ: 1.25 (t, 6H,

OCH_2CH_3, $^3J_{HH}$ = 7.0 Hz), 1.88 (dt, 2H, CH_2CH_2P, $^2J_{PH}$ = 18.0 Hz, $^3J_{HH}$ = 8.0 Hz), 2.86 (m, 2H, CH_2CH_2P), 3.82 (s, 4H, cyclic NCH_2), 4.03 (m, 4H, OCH_2CH_3), 7.50–7.55 (d, 4H, aromatic), 7.80 (s, 2H, CH=), 8.26–8.30 (d, 4H, aromatic). ^{13}C ($CDCl_3$), δ: 16.23 (d, $POCH_2CH_3$, $^3J_{CP}$ = 6.0 Hz), 24.08 (d, NCH_2CH_2P, J_{CP} = 139 Hz), 50.54 (NCH_2CH_2P), 53.98 ($N(CH_2)_2$ cyclic), 61.57 (d, $POCH_2CH_3$, $^2J_{CP}$ = 6.4 Hz), 123.70 (C(8), C(10), C(8′), C(10′)), 130.67 (C(7), C(11), C(7′), C(11′)), 134.17 (C(5) and C(5′)), 135.18 (C(3) and C(3′)), 140.93 (C(6) and C(6′)), 147.42 (C(9) and C(9′), 185.88 (C(O)). ^{31}P ($CDCl_3$), δ: 30.53. HRMS calculated for $C_{25}H_{28}N_3O_8P$ (M^+) 529.16140, found 529.16073; calculated for $[M–OH]^+$ 512.15866, found 512.15818.

4.7.7. Diethyl 3-[3,5-bis(4-dimethylaminobenzylidene)-4-oxopiperidin-1-yl]-propylphosphonate **7g**

Gradient elution starting from CH_2Cl_2 to CH_2Cl_2/acetone = 5:1; crystallization from $CHCl_3$/hexane mixture. Orange-red crystal solid, m.p. 140–142 °C. IR: 1657 (C=O), 1587, 1524, 1444, 1372, 1308, 1233, 1160, 1063 (P–O–C), 1025 (P–O–C), 988, 948, 814, 514. ^{1}H ($CDCl_3$), δ: 1.20 (t, 6H, $POCH_2CH_3$, $^3J_{HH}$ = 7.1 Hz), 1.75 (m, 4H, $NCH_2CH_2CH_2P$), 2.62 (t, 2H, $NCH_2CH_2CH_2P$, $^3J_{HH}$ = 6.4 Hz), 3.01 (s, 12H, $2NMe_2$), 3.83 (s, 4H, cyclic $N(CH_2)_2$), 3.98 (m, 4H, $POCH_2CH_3$), 6.70 (d, 4H aromatic, $^3J_{FH}$ = 8.9 Hz), 7.33 (d, 4H aromatic, J_{HH} = Hz), 7.74 (s, 2H, CH=). ^{13}C ($CDCl_3$), δ: 16.14 (d, $POCH_2CH_3$, $^3J_{CP}$ = 5.9 Hz), 20.12 (d, $NCH_2CH_2CH_2P$, $^2J_{CP}$ = 4.4 Hz), 22.78 (d, $NCH_2CH_2CH_2P$, J_{CP} = 141.2 Hz), 39.84 (NMe_2), 54.78 (cyclic $N(CH_2)_2$), 57.04 (d, $NCH_2CH_2CH_2P$, $^3J_{CP}$ = 17.6 Hz), 61.18 (d, $POCH_2CH_3$, $^2J_{CP}$ = 6.6 Hz), 111.48 (C(8), C(10), C(8′), C(10′)), 123.10 (C(6) and C(6′)), 129.03 (C(3) and C(3′)), 132.27 (C(7), C(11), C(7′), C(11′)), 136.30 (C(5) and C(5′)), 150.33 (C(9) and C(9′)), 186.76 (C(O)). ^{31}P ($CDCl_3$), δ: 32.20. Anal. calcd. for $C_{30}H_{42}N_3O_4P$: C 66.77, H 7.84, N 7.79%; found: C 66.64, H 7.97, N 7.81%.

4.7.8. Diethyl 3-[3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl]propylphosphonate **7h**

Gradient elution starting from CH_2Cl_2 to CH_2Cl_2/acetone = 5:1. Yellow crystal solid, m.p. 84–90 °C (no sharp melting). IR: 1673 (C=O), 1614, 1599, 1577, 1508, 1293, 1266, 1227, 1183, 1161, 1051 (P–O–C), 1034 (P–O–C), 1021, 967, 948, 908, 803, 780, 569, 533, 497. ^{1}H ($CDCl_3$), δ: 1.18 (t, 6H, $POCH_2CH_3$, $^3J_{HH}$ = 7 Hz), 1.69 (m, 4H, $NCH_2CH_2CH_2P$), 2.61 (m, 2H, $NCH_2CH_2CH_2P$), 3.78 (s, 4H, cyclic $N(CH_2)_2$), 3.96 (m, 4H, $POCH_2CH_3$), 7.09 (t, 4H, C(10)–H, C(8)–H, C(10′)–H, C(8′)–H, $^3J_{FH}$ = 8.6 Hz, $^3J_{HH}$ = 8.6 Hz), 7.35 (dd, C(7)–H, C(11)–H, C(7′)–H, C(11′)–H, $^3J_{HH}$ = 8.6 Hz, $^4J_{FH}$ = 5.4 Hz), 7.75 (s, 2H, CH=). ^{13}C ($CDCl_3$), δ: 16.30 (d, $POCH_2CH_3$, $^3J_{CP}$ = 6.6 Hz), 20.18 (d, $NCH_2CH_2CH_2P$, $^2J_{CP}$ = 4.4 Hz), 22.94 (d, $NCH_2CH_2CH_2P$, J_{CP} = 141.5 Hz), 54.48 (cyclic $N(CH_2)_2$), 57.00 (d, $NCH_2CH_2CH_2P$, $^3J_{CP}$ = 16.9 Hz), 61.41 (d, $POCH_2CH_3$, $^2J_{CP}$ = 6.6 Hz), 115.75 (d, C(8), C(10), C(8′), C(10′), $^2J_{CF}$ = 21.3 Hz), 131.26 (d, C(6) and C(6′), $^4J_{CF}$ = 3.7 Hz), 132.72 (C(3) and C(3′)), 132.31 (d, C(7), C(11), C(7′), C(11′), $^3J_{CF}$ = 8 Hz), 135.26 (C(5) and C(5′)), 162.90 (d, C(9) and C(9′), J_{CF} = 251 Hz), 186.91 (C(4)). ^{19}F ($CDCl_3$), δ: −110.66. ^{31}P ($CDCl_3$), δ: 31.88. Anal. calcd. for $C_{26}H_{30}F_2NO_4P$: C 63.80, H 6.18, N 2.86%; found: C 63.85, H 6.13, N 2.77%.

4.7.9. Diethyl 3-[3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl]-propylphosphonate **7i**

Gradient elution starting from CH_2Cl_2 to CH_2Cl_2/acetone = 5:1; crystallization from CH_2Cl_2/Et_2O mixture. Yellow crystal solid, m.p. 133–136 °C. IR: 1678 (C=O), 1618, 1598, 1592, 1514, 1348, 1339, 1308, 1279, 1260, 1247, 1234, 1204, 1180, 1120, 1110, 1099, 1057 (P–O–C), 1027 (P–O–C), 1008, 966, 947, 926, 859, 810, 759. ^{1}H ($CDCl_3$), δ: 1.19 (t, 6H, $POCH_2CH_3$, $^3J_{HH}$ = 7.1 Hz), 1.65 (m, 4H, $NCH_2CH_2CH_2P$), 2.62 (t, 2H, $NCH_2CH_2CH_2P$, $^3J_{HH}$ = 6.7 Hz), 3.80 (s, 4H, cyclic $N(CH_2)_2$), 3.96 (m, 4H, $POCH_2CH_3$), 7.52 (4H aromatic), 7.79 (s, 2H, CH=), 8.27 (4H aromatic). ^{13}C ($CDCl_3$), δ: 16.09 (d, $POCH_2CH_3$, $^3J_{CP}$ = 6.2 Hz), 19.87 (d, $NCH_2CH_2CH_2P$, $^2J_{CP}$ = 4.4 Hz), 22.69 (d, $NCH_2CH_2CH_2P$, J_{CP} = 142.3 Hz), 54.19 ($N(CH_2)_2$ cyclic), 56.84 (d, $NCH_2CH_2CH_2P$, $^3J_{CP}$ = 17.2 Hz), 61.19 (d, $POCH_2CH_3$, $^2J_{CP}$ = 6.2 Hz), 123.56 (C(8), C(10), C(8′), C(10′)), 130.60 (C(7), C(11), C(7′), C(11′)), 133.78 (C(5) and C(5′)), 135.52 (C(3) and C(3′)), 140.99 (C(6) and C(6′)), 147.28 (C(9) and C(9′)), 186.10 (C(O)). ^{31}P ($CDCl_3$), δ: 31.59. Anal. calcd. for $C_{26}H_{30}N_3O_8P$: C 57.46, H 5.56, N 7.73%; found: C 57.46, H 5.52, N 7.57%.

4.7.10. Diethyl 3-[3,5-bis(4-dimethylaminobenzylidene)-4-oxopiperidin-1-yl]-butylphosphonate **7j**

Gradient elution starting from $CHCl_3$ to $CHCl_3$/acetone = 10:1; crystallization from CH_2Cl_2/hexane mixture. Orange-red crystal solid, m.p. 146–148 °C. ^{1}H ($CDCl_3$), δ: 1.25 (t, 6H, $POCH_2CH_3$, $^3J_{HH}$ = 7.1 Hz), 1.63 (m, 6H, $NCH_2CH_2CH_2P$), 2.55 (t, 2H, $NCH_2CH_2CH_2CH_2P$, $^3J_{HH}$ = 7.1 Hz), 3.00 (12H, $2NMe_2$), 3.80 (s, 4H, $N(CH_2)_2$ cyclic), 4.01 (m, 4H, $POCH_2CH_3$), 6.69 (4H aromatic), 7.32 (4H aromatic), 7.73 (s, 2H, CH=). ^{13}C ($CDCl_3$), δ: 16.03 (d, $POCH_2CH_3$, $^3J_{CP}$ = 6.3 Hz), 19.97 (d, $NCH_2CH_2CH_2CH_2P$, $^2J_{CP}$ = 4.8 Hz), 25.01 (d, $NCH_2CH_2CH_2CH_2P$, $^1J_{CP}$ = 140.4 Hz), 27.79 (d, $NCH_2CH_2CH_2CH_2P$, $^3J_{CP}$ = 16.3 Hz), 39.68 (NMe_2), 54.78 ($N(CH_2)_2$ cyclic), 56.32 ($NCH_2CH_2CH_2CH_2P$), 60.97 (d, $POCH_2CH_3$, $^2J_{CP}$ = 6.3 Hz), 111.30 (C(8), C(10), C(8′), C(10′)), 123.04 (C(6) and C(6′)), 128.98 (C(3) and C(3′)), 132.09 (C(7), C(11), C(7′), C(11′)), 136.10 (C(5) and C(5′)), 150.13 (C(9) and C(9′)), 186.63 (C(O)). ^{31}P ($CDCl_3$), δ: 32.08. Anal. calcd. for $C_{31}H_{44}N_3O_4P$: C 67.25, H 8.01, N 7.59%; found: C 67.29, H 8.01, N 7.64%.

4.7.11. Diethyl 3-[3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl]-butylphosphonate **7k**

Gradient elution starting from CH_2Cl_2 to CH_2Cl_2/acetone = 5:1. Yellow crystal solid, m.p. 117–119 °C. ^{1}H ($CDCl_3$), δ: 1.24 (t, 6H, $POCH_2CH_3$, $^3J_{HH}$ = 7.1 Hz), 1.60 (m, 6H, $NCH_2CH_2CH_2CH_2P$), 2.54 (t, 2H, $NCH_2CH_2CH_2CH_2P$, $^3J_{HH}$ = 6.6 Hz), 3.78 (s, 4H, cyclic $N(CH_2)_2$), 4.01 (m, 4H, $POCH_2CH_3$), 7.53 (4H aromatic), 7.79 (s, 2H, CH=), 8.28 (4H aromatic). ^{13}C ($CDCl_3$), δ: 16.17 (d, $POCH_2CH_3$, $^3J_{CP}$ = 5.1 Hz), 19.95 (d, $NCH_2CH_2CH_2CH_2P$, $^2J_{CP}$ = 3.7 Hz), 25.05 (d, $NCH_2CH_2CH_2CH_2P$, $^1J_{CP}$ = 141.2 Hz), 27.51 (d, $NCH_2CH_2CH_2CH_2P$, $^3J_{CP}$ = 16.1 Hz), 54.36 ($N(CH_2)_2$ cyclic), 56.44 ($NCH_2CH_2CH_2CH_2P$), 61.21 (d, $POCH_2CH_3$, $^2J_{CP}$ = 6.2 Hz), 123.60 (C(8), C(10), C(8′), C(10′)), 130.62 (C(7), C(11), C(7′), C(11′)), 133.79 (C(5) and C(5′)), 135.63 (C(3) and C(3′)), 141.08 (C(6) and C(6′)), 147.31 (C(9) and C(9′)), 186.19 (C(O)). ^{31}P ($CDCl_3$), δ: 31.76. Anal. calcd. for $C_{27}H_{32}N_3O_8P$: C 58.17, H 5.79, N 7.54%; found: C 58.17, H 5.71, N 7.39%.

4.8. 3-[3,5-Bis(substituted benzylidene)-4-oxopiperidin-1-yl]alkyl phosphonic acids **9a–g** (general procedure)

To a stirred solution of the corresponding diethyl phosphonate (2 mmol) **7** dissolved in dry $CHCl_3$ (10 mL) a solution of bromotrimethylsilane (8 mmol) in dry $CHCl_3$ (3 mL) was added at one portion from syringe at room temperature. In the case of compounds **7d**,**g** bearing the dimethylamino group an excess of bromotrimethylsilane was used (28 mmol). Reaction solution was stirred over 24 h at room temperature. The acids **9c**,**f** precipitated from the reaction solution and in this case the stirring over 3 days was necessary to complete the transformation. $CHCl_3$ was removed at reduced pressure and the solid remaining was treated with a mixture of MeOH:H_2O = 10:1 (v/v). Insoluble product was filtered off, washed with a small portion of MeOH, then Et_2O and dried under P_2O_5 at 1 mm Hg to afford analytical pure compound. In the case of acids **8c**,**f** which were soluble in MeOH, methanol was removed to dryness to give the desired compound.

4.8.1. [(3E,5E)-3,5-Bis(4-fluorobenzylidene)-4-oxo-1-piperidinyl]methylphosphonic acid **9a**

Yield 89%, decompose above 235 °C. Light-yellow crystal solid. ^{1}H (DMSO-d_6), δ: 2.84 (d, 2H, CH_2P, $^2J_{PH}$ = 12 Hz), 4.07 (broad s, 24H,

cyclic NCH_2 + H_2O + P–OH), 7.29 (t, 4H, $^3J_{HH}$ = 8 Hz, $^3J_{FH}$ = 8 Hz), 7.57 (dd, 4H, $^3J_{HH}$ = 8 Hz, $^4J_{FH}$ = 5 Hz), 7.60 (s, 2H, CH=). ^{19}F (DMSO-d_6), δ: −111.50. ^{31}P (DMSO-d_6), δ: 19.26. Anal. calcd. for $C_{20}H_{18}F_2NO_4P \cdot 1/2H_2O$: C 57.98, H 4.62, N 3.38%; found: C 57.95, H 4.45, N 3.37%.

4.8.2. [(3E,5E)-3,5-Bis(4-nitrobenzylidene)-4-oxo-1-piperidinyl]methylphosphonic acid **9b**

Yield 93%, decompose >200 °C. Yellow crystal solid. ^{1}H (DMSO-d_6), δ: 2.85 (d, 2H, CH_2P, $^2J_{PH}$ = 12 Hz), 4.12 (s, 4H, NCH_2 cyclic), 6.15 (broad s, 4H, 0.5H_2O + P–OH), 7.68–7.73 (m, 4H, aromatic), 7.77 (s, 2H, CH=), 8.23–8.28 (m, 4H, aromatic). ^{31}P (DMSO-d_6), δ: 19.16. Anal. calcd. for $C_{20}H_{18}N_3O_8P$: C 52.30, H 3.95, N 9.15%; found: C 51.96, H 3.85, N 9.11%.

4.8.3. 2-[(3E,5E)-3,5-Bis(4-(dimethylamino)benzylidene)-4-oxo-1-piperidinyl]-ethylphosphonic acid hydrobromide **9c**

Yield 72%. Dark-red crystal solid. ^{1}H (DMSO-d_6), δ: 2.02–2.20 (m, 2H, CH_2CH_2P), 3.03 (s, 12H, NMe_2), 3.50 (m, 2H, CH_2CH_2P), 4.66 (s, 4H, NCH_2 cyclic), 6.54 (broad s, 11H, $3H_2O$ + acid protons), 6.86–6.90 (m, 4H, aromatic), 7.44–7.48 (m, 4H, aromatic), 7.77 (s, 2H, CH=). ^{31}P (DMSO-d_6), δ: 20.96. Anal. calcd. for $C_{25}H_{32}N_3O_4P \cdot 2.9HBr \cdot 1/2H_2O$: C 42.10, H 5.08, Br 32.49, N 5.89%; found: C 42.28, H 4.65, N 2.91%.

4.8.4. 2-[(3E,5E)-3,5-Bis(4-fluorobenzylidene)-4-oxo-1-piperidinyl]ethylphosphonic acid hydrobromide **9d**

Yield 43%, m.p. 213–216 °C (decompose). Light-yellow crystal solid. ^{1}H (DMSO-d_6), δ: 1.82–1.95 (m, 2H, CH_2CH_2P), 3.08 (m, 2H, CH_2CH_2P), 4.17 (s, 4H, NCH_2 cyclic), 4.52 (broad s, 13H, $5H_2O$ + P–OH), 7.33 (t, 4H, $^3J_{HH}$ = 8 Hz, $^3J_{FH}$ = 8 Hz), 7.63 (dd, 4H, $^3J_{HH}$ = 8 Hz, $^4J_{FH}$ = 5 Hz), 7.72 (s, 2H, CH=). ^{19}F (DMSO-d_6), δ: −110.87. ^{31}P (DMSO-d_6), δ: 23.34. Anal. calcd. for $C_{21}H_{20}F_2NO_4P \cdot 0.625HBr$: C 53.61, H 4.41, N 2.99%; found: C 53.45, H 4.95, Br 32.47, N 5.66%.

4.8.5. 2-[(3E,5E)-3,5-Bis(4-nitrobenzylidene)-4-oxo-1-piperidinyl]ethylphosphonic acid hydrobromide **9e**

Yield 96%, decompose > 200 °C. Yellow crystal solid. ^{1}H (DMSO-d_6), δ: 1.79–1.97 (m, 2H, CH_2CH_2P), 3.09 (m, 2H, CH_2CH_2P), 4.28 (broad s, 17H, NCH_2 cyclic and acid protons), 7.78–7.82 (m, 6H, aromatic + CH=), 8.26–8.31 (m, 4H, aromatic). ^{31}P (DMSO-d_6), δ: 23.11. Anal. calcd. for $C_{21}H_{20}N_3O_8P \cdot 2/3H_2O \cdot 1/2HBr$: C 47.97, H 4.19, Br 7.60, N 7.99%; found: C 47.98, H 4.04, Br 7.64, N 7.84%.

4.8.6. 3-[(3E,5E)-3,5-Bis[4-(dimethylamino)benzylidene]-4-oxo-1-piperidinyl]propylphosphonic acid hydrobromide **9f**

Yield 80%. Dark-red crystal solid. ^{1}H (DMSO-d_6), δ: 1.61 (m, 2H, $CH_2CH_2CH_2P$), 1.90 (m, 2H, $CH_2CH_2CH_2P$), 3.05 (s, 12H, NMe_2), 3.45 (t, 2H, $CH_2CH_2CH_2P$, $^3J_{HH}$ = 7.5 Hz), 4.65 (broad s overlapped with protons of water, NCH_2 cyclic), 6.90–6.92 (m, 4H, aromatic), 7.45–7.47 (m, 4H, aromatic), 7.81 (s, 2H, CH=). ^{31}P (DMSO-d_6), δ: 24.98. This compound was transformed into the corresponding sodium salt without additional purification.

4.8.7. 3-[(3E,5E)-3,5-Bis(4-nitrobenzylidene)-4-oxo-1-piperidinyl]propylphosphonic acid hydrobromide **9g**

Yield 81%. Yellow crystal solid. ^{1}H (DMSO-d_6), δ: 1.55 (m, 2H, $CH_2CH_2CH_2P$), 1.82 (m, 2H, $CH_2CH_2CH_2P$), 3.28 (t, 2H, $CH_2CH_2CH_2P$, $^3J_{HH}$ = 7.1 Hz), 4.58 (s, 4H, NCH_2 cyclic), 7.83–7.85 (m, 4H, aromatic), 7.98 (s, 2H, CH=), 8.33–8.35 (m, 4H, aromatic). ^{31}P (DMSO-d_6), δ: 24.98. Anal. calcd. for $C_{22}H_{22}N_3O_8P \cdot 1.83H_2O \cdot 0.83H$ Br: C 44.95, H 4.54, Br 11.33, N 7.15%; found: C 44.64, H 4.07, Br 11.45, N 7.51%.

4.9. [3E,5E-Bis(benzylidene)-4-oxopiperidin-1-yl]alkylphosphonic acid disodium salts **10** (general procedure)

A filtered solution of sodium hydroxide (0.16 g, 0.004 mol) in EtOH (10 mL) was added to a stirred powder of the corresponding acid **9** (0.001 mol). The reaction mixture was stirred at room temperature for 24 h. Then the solid product was filtered off, washed several times with anhydrous EtOH, Et_2O and air-dried.

4.9.1. Disodium [3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl]methylphosphonate **10a**

Yield 79%. Light-yellow crystal solid. ^{1}H (D_2O), δ: 2.63 (d, 2H, CH_2P, $^2J_{PH}$ = 12 Hz), 3.94 (s, 4H, NCH_2 cyclic), 7.10 (t, 4H, $^3J_{HH}$ = 8 Hz, $^3J_{FH}$ = 8 Hz), 7.41 (dd, 4H, $^3J_{HH}$ = 8 Hz, $^4J_{FH}$ = 5 Hz), 7.52 (s, 2H, CH=). ^{19}F (D_2O), δ: −111.26. ^{31}P (D_2O), δ: 15.18. Anal. calcd. for $C_{20}H_{16}F_2NNa_2O_4P \cdot 6H_2O$: C 43.10, H 5.06, N 2.51%; found: C 42.98, H 4.81, N 2.39%.

4.9.2. Disodium [3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl]methylphosphonate **10b**

Yield 63%, yellow crystal solid. ^{1}H (D_2O), δ: 2.67 (d, 2H, CH_2P, $^2J_{PH}$ = 12 Hz), 4.03 (s, 4H, NCH_2 cyclic), 7.49–7.53 (m, 6H, aromatic + CH=), 8.09–8.12 (m, 4H, aromatic). ^{31}P (D_2O), δ: 15.16. Anal. calcd. for $C_{20}H_{16}N_3Na_2O_8P \cdot 2H_2O$: C 44.54, H 3.74, N 7.79%; found: C 44.67, H 3.58, N 7.67%.

4.9.3. Disodium 2-[3,5-bis(4-(dimethylamino)benzylidene)-4-oxopiperidin-1-yl]ethylphosphonate **10c**

Yield 89%, decompose above 200 °C. Dark-red crystal solid. ^{1}H (D_2O), δ: 1.58 (m, 2H, CH_2CH_2P), 2.84 (m, 14H, CH_2CH_2P and NMe_2), 3.62 (s, 4H, NCH_2 cyclic), 6.72–6.76 (m, 4H, aromatic), 7.27–7.31 (m, 4H, aromatic), 7.50 (s, 2H, CH=). ^{13}C (D_2O/DMSO-d_6), δ: 27.87 (d, NCH_2CH_2P, J_{CP} = 125 Hz), 39.56 (NCH_2CH_2P), 41.21 (NMe_2), 54.83 ($N(CH_2)_2$ cyclic), 114.24 (C(8), C(10), C(8′), C(10′)), 124.31 (C(6) and C(6′)), 129.29 (C(7), C(11), C(7′), C(11′)), 135.23 (C(5) and C(5′)), 140.49 (C(3) and C(3′)), 153.26 (C(9) and C(9′)), 189.54 (C(4)). ^{31}P (D_2O), δ: 20.03. Anal. calcd. for $C_{25}H_{30}N_3Na_2O_4P \cdot 1.5H_2O$: C 55.55, H 6.15, N 7.77%; found: C 55.07, H 5.81, N 7.61%.

4.9.4. Disodium 2-[3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl]ethylphosphonate **10d**

Yield 100%, decompose above 200 °C. Light-yellow crystal solid. ^{1}H (D_2O), δ: 1.50–1.65 (m, 2H, CH_2CH_2P), 2.78 (m, 2H, CH_2CH_2P), 3.78 (s, 4H, NCH_2 cyclic), 7.14 (t, 4H, $^3J_{HH}$ = 8 Hz, $^3J_{FH}$ = 8 Hz), 7.44 (m, 4H, aromatic), 7.62 (s, 2H, CH=). ^{13}C(D_2O): 25.91 (d, NCH_2CH_2P, J_{CP} = 124 Hz), 53.00 (NCH_2CH_2P and $N(CH_2)_2$ cyclic, $^2J_{CP}$ = 11 Hz), 115.62 (d, C(8), C(10), C(8′), C(10′), $^2J_{CF}$ = 21.9 Hz), 130.27 (s, C(6) and C(6′)), 131.25 (C(3) and C(3′)), 133.22 (d, C(7), C(11), C(7′), C(11′), $^3J_{CF}$ = 8.4 Hz), 137.40 (C(5) and C(5′)), 163.03 (d, C(9) and C(9′), J_{CF} = 250 Hz), 188.60 (C(4)). ^{19}F (D_2O), δ: −111.12. ^{31}P (D_2O), δ: 20.01. Anal. calcd. for $C_{21}H_{18}F_2NNa_2O_4P \cdot 2.5H_2O$: C 49.62, H 4.56, N 2.76%; found: C 49.64, H 3.81, N 2.81%.

4.9.5. Disodium 2-[3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl]ethylphosphonate **10e**

Yield 100%, decompose above 200 °C. Yellow crystal solid. ^{1}H (D_2O), δ: 1.53 (m, 2H, CH_2CH_2P), 2.75 (m, 2H, CH_2CH_2P), 3.75 (s, 4H, NCH_2 cyclic), 7.43–7.46 (m, 4H, aromatic), 7.47 (s, 2H, CH=), 8.03–8.08 (m, 4H, aromatic). ^{13}C (D_2O/DMSO-d_6), δ: 25.83 (d, NCH_2CH_2P, J_{CP} = 125 Hz), 37.92 (NCH_2CH_2P), 53.08 ($N(CH_2)_2$ cyclic), 123.71 (C(8), C(10), C(8′), C(10′)), 131.70 (C(7), C(11), C(7′), C(11′)), 134.66 (C(5) and C(5′)), 135.57 (C(3) and C(3′)), 140.45 (C(6) and C(6′)), 147.17 (C(9) and C(9′), 187.56 (C(O)). ^{31}P (D_2O), δ: 20.02. Anal. calcd. for $C_{21}H_{18}N_3Na_2O_8P \cdot H_2O$: C 47.11, H 3.77, N 7.85%; found: C 47.14, H 3.61, N 7.94%.

4.9.6. Disodium 2-[3,5-bis(4-(dimethylamino)benzylidene)-4-oxopiperidin-1-yl]propylphosphonate **10f**

Yield 63% (based on starting ester **7g**), decompose above 200 °C. Dark-red crystal solid. ^{1}H (D_2O), δ: 1.23 (m, 2H, $CH_2CH_2CH_2P$), 1.53 (m, 2H, $CH_2CH_2CH_2P$), 2.46 (m, 2H, $CH_2CH_2CH_2P$), 2.75 (s, 12H,

NMe_2), 3.50 (s, 4H, NCH_2 cyclic), 6.61–6.63 (m, 4H, aromatic), 7.16–7.18 (m, 4H, aromatic), 7.43 (s, 2H, CH=). ^{13}C: (D_2O), δ: 21.51 ($NCH_2\underline{C}H_2CH_2P$), 27.21 (d, $NCH_2CH_2\underline{C}H_2P$, $J_{CP} = 131$ Hz), 39.54 (NMe_2), 53.57 ($N(CH_2)_2$ cyclic), 58.4 (d, $N\underline{C}H_2CH_2CH_2P$, $J_{CP} = 20.5$ Hz), 112.58 (C(8), C(10), C(8′), C(10′)), 122.68 (C(6) and C(6′)), 127.33 (C(7), C(11), C(7′), C(11′)), 133.49 (C(5) and C(5′)), 139.13 (C(3) and C(3′)), 151.61 (C(9) and C(9′)), 187.88 (C(4)). ^{31}P (D_2O), δ: 21.64. Anal. calcd. for $C_{26}H_{32}N_3Na_2O_4P \cdot 3.5H_2O$: C 52.88, H 6.66, N 7.12%; found: C 52.55, H 6.13, N 7.07%.

4.9.7. Disodium 2-[3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl]propylphosphonate **10g**

Yield 95%, decompose above 200 °C. Yellow crystal solid. ^1H (D_2O), δ: 1.25 (m, 2H, $CH_2CH_2\underline{CH_2}P$), 1.57 (m, 2H, $CH_2\underline{CH_2}CH_2P$), 2.56 (t, 2H, $\underline{CH_2}CH_2CH_2P$, $^3J_{HH} = 7$ Hz), 3.76 (s, 4H, NCH_2 cyclic), 7.44–7.47 (m, 6H, aromatic + CH=), 8.06–8.07 (m, 4H, aromatic). ^{31}P (D_2O), δ: 21.69. IR: 3108, 1677, 1616, 1599, 1593, 1516, 1344, 1315, 1259, 1176, 1109, 1058 (strong, broad), 980, 855, 808, 757, 713, 688. Anal. calcd. for $C_{22}H_{20}N_3Na_2O_8P \cdot 4H_2O$: C 43.79, H 4.68, N 6.96%; found: C 43.53, H 4.42, N 6.68%.

4.10. Biological evaluations

Cell lines used for estimation of toxicity of compounds **7a**–**k** and **10a**–**g** were CaOv3 and Scov3 (human ovarian carcinoma), PC3 (human prostate carcinoma) and A549 (human lung carcinoma). Cells were grown in RPMI-1640 medium (Sigma–Aldrich, UK) supplemented with 10% fetal bovine serum (FBS, HyClone, USA), 2 mM L-glutamine and gentamicin. Cytotoxicity of the individual compounds was measured for each cell line after 72 h of cultivation by the MTT (3-(4,5-dimethyldiazolyl-2)-2,5-diphenyl tetrazolium-bromide) colorimetric assay. The test is based on the ability of mitochondrial dehydrogenase in viable cells to convert MTT reagent (ICN Biomedicals, Germany) into a soluble blue formazan dye. Briefly, the different cell lines were seeded into 96-well plates at a concentration of 1×10^4 cells/100 μL/well. The cells were allowed to attach overnight at 37 °C in a humidified atmosphere containing 5% CO_2. The tested compounds were initially dissolved in dimethylsulfoxide (DMSO) and the working solutions were added to FBS free culture medium. The compounds were added to wells with increasing drug concentrations. After 72 h incubation, 20 μL of MTT reagent (5 mg/mL) were added and cell cultures were incubated for 3 h at 37 °C. After removal of the culture medium formazan crystals were dissolved in dimethylsulfoxide (Sigma–Aldrich) to determine the amount of formazan product. The optic density (OD) was determined by the multi-well plate reader (Uniplan, Picon, Russia) at 590 nm. The results were expressed as percent decrease of cell viability as compared to untreated controls. Each concentration of the compound tested was examined in triplicate and the IC_{50} values were determined graphically. The concentrations of compounds used were 5×10^{-5}, 10^{-5}, 10^{-6}, 10^{-7} M. Commercially available Melphalan (Sarcolysin) purchased from Arkelan–Glaxo was used as a positive control in the assay.

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Appendix. Supplementary information

Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.ejmech.2008.10.019.

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