Med Chem Res
DOI 10.1007/s00044-016-1726-4

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

1,5-Diaryl-3-oxo-1,4-pentadienes based on (4-oxopiperidin-1-yl)(aryl)methyl phosphonate scaffold: synthesis and antitumor properties

Mikhail V. Makarov[1] · **Ekaterina Yu. Rybalkina**[2] · **Lada V. Anikina**[3] · **Sergey A. Pukhov**[3] · **Sergey G. Klochkov**[3] · **Denis V. Mischenko**[4] · **Margarita E. Neganova**[3] · **Victor N. Khrustalev**[1,5] · **Zinaida S. Klemenkova**[1] · **Valery K. Brel**[1]

Received: 19 March 2016 / Accepted: 23 September 2016

Abstract Novel 3,5-bis(arylidene)-4-piperidones modified with diethyl[(aryl)methyl]phosphonate moiety attached to the piperidone nitrogen atom have been synthesized by crotonic condensation of aromatic aldehydes with diethyl [(4-oxopiperidin-1-yl)(aryl)methyl]phosphonates in the presence of $LiClO_4/Et_3N$ system or acetonitrile solution of boron trifluoride etherate. The synthesized phosphonate derivatives of 3,5-bis(arylidene)-4-piperidone series displayed inhibitory properties toward RD, PC3, HCT116, and MCF7 human cancer cell lines with IC_{50} values in the range of 2.5–8.5 μM, as assessed by an in vitro 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Keywords 3,5-Bis(arylidene)-4-piperidones · Antitumor activity · Aminophosphonates · Aryl(methyl) phosphonates · 1,5-Diaryl-3-oxo-1,4-pentadienes

Introduction

Linear (**A**) and cyclic (**B**) 1,5-diaryl-3-oxo-1,4-pentadienes are considered as synthetic analogues of naturally occurring compound curcumin (Fig. 1) distinguished by pronounced anticancer activity and simultaneously exhibiting low acute toxicity (Shehzad et al. 2010). These cross-conjugated pentadienones have been proposed to overcome some of the pharmacological drawbacks of curcumin (Anand et al. 2007), such as its low solubility and bioavailability as well as low chemical stability in aqueous media at physiological pH, and to develop novel drug candidates with higher antitumor activity or selectivity to malignant cells (Mosley et al. 2007; Kudo et al. 2011; Shibata and Iwabuchi 2012; Vyas et al. 2013; Shetty et al. 2015).

An efficient approach for regulating the biological activity of the dienones consists in varying substituents (R^1 and R^2 in **A** and **B**, Fig. 1) in their aromatic rings (Gregory et al. 2013) owing to a strong influence of these substituents on the electrophilic properties of the conjugated vinyl bonds, which are capable of reacting with cellular thiols while leaving intact nitrogen nucleophiles such as nucleotides.

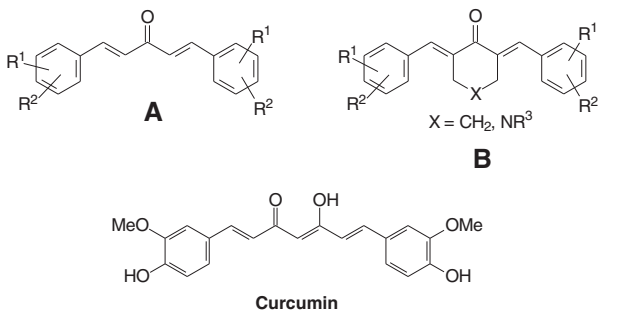


Fig. 1 Structures of acyclic and cyclic 1,5-diaryl-3-oxo-1,4-pentadienes and curcumin

Electronic supplementary material The online version of this article (doi:10.1007/s00044-016-1726-4) contains supplementary material, which is available to authorized users.

✉ Mikhail V. Makarov
mmak78@yandex.ru

[1] Nesmeyanov Institute of Organoelement Compounds, RAS, Moscow 119991, Russian Federation

[2] Institute of Carcinogenesis, Blokhin Russian Cancer Research Center, Moscow 115478, Russian Federation

[3] Institute of Physiologically Active Substances, RAS, Chernogolovka 142432, Russian Federation

[4] Institute of Problems of Chemical Physics, RAS, Chernogolovka 142432, Russian Federation

[5] Peoples' Friendship University of Russia, Moscow 117198, Russian Federation

Published online: 01 November 2016

For example, it was shown that vinyl bonds of the dienone scaffold may covalently bind with sulfhydryl group of cysteine 88 of ubiquitin receptor RPN13, which resulted in inhibition of proteasome function and accumulation of polyubiquitinated proteins in cancer cells and finally in their apoptosis (Anchoori et al. 2013). In view of this, a hypothesis, which states that an increase in the electrophilic character of aromatic rings in the structure of 1,5-diaryl-3-oxo-1,4-pentadienes might result in their higher cytotoxicity, seems to be rational (Das et al. 2009a). Indeed, some dienone compounds containing electron-withdrawing nitro groups in aromatic rings were shown to exhibit high inhibitory activity toward various malignant cell lines. Based on this observation, a model for a hypothetical receptor active site for this class of compounds was proposed (Patel et al. 2007). On the other hand, parent curcumin molecule contains electron-releasing hydroxy and methoxy substituents on phenyl rings and there were published examples of monocarbonyl curcumin analogues having only methoxy groups as substituents and revealing much higher anticancer activity than curcumin (Gregory et al. 2013; Shetty et al. 2015; Youssef and El-Sherbeny 2005). It should be also pointed out that dienones have a variety of molecular targets that may be differently affected by these compounds depending on their structural features, such as location and electronic properties of substituents in the aromatic moieties (Das et al. 2009a). Therefore, influence of these substituents on the antitumor properties of dienone curcumin analogues is of a complex nature and needs further investigation.

Another approach that is available in the case of dienones based on 4-piperidone framework (compounds of 3,5-bis(arylidene)-4-piperidone series) includes attaching various groups (R^3 in **B**, Fig. 1) to piperidone nitrogen atom. These groups may possess inherent biological activity, improve pharmacological properties of the dienone pharmacophore or facilitate interaction with a receptor binding site. Moreover, combining the dienone moiety and such groups in one molecule may result in a synergistic increase in cytotoxicity toward cancer cells as envisaged, for example, by the theory of sequential cytotoxicity (Das et al. 2009a). Examples of such modifying groups include acryloyl group (Dimmock et al. 2001), residues of *N*-aryl fumaramic acids (Jha et al. 2007), amino acids (Bazzaro et al. 2011) and aromatic sulfonic acids (Thakur et al. 2014; Sun et al. 2014), as well as nitroxides (Kálai et al. 2011) or even another 3,5-bis(arylidene)-4-piperidone scaffold attached through an appropriate linker (Das et al. 2011, 2013; Santiago-Vazquez et al. 2014).

Organophosphorus moieties, such as phosphonate, bisphosphonate and phosphate residues, have also found application as structural modifiers of 3,5-bis(arylidene)-4-piperidones. For instance, it was shown that the direct phosphorylation of *NH*-3,5-bis(arylidene)-4-piperidones ($R^3 = H$ in Fig. 1) with diethyl chlorophosphate provided corresponding amides ($R^3 = P(O)(OEt)_2$), with some of them revealing higher anticancer activity than non-phosphorylated *NH*-analogues (Das et al. 2009b). Moreover, these amides exhibited higher cytotoxicity toward cancer cells as compared to normal cells (Das et al. 2010). Another advantageous feature of *N*-phosphorylated 3,5-bis(arylidene)-4-piperidones is their good membrane permeability (Singh et al. 2014). High cytotoxicity toward malignant cells was also demonstrated by 3,5-bis(arylidene)-4-piperidones bearing diethyl phosphonate and tetraethyl (methylene)bisphosphonate groups attached to the piperidone nitrogen atom through short alkylene chains (Makarov et al. 2009; Makarov et al. 2012). Therefore, synthesis and investigation of antitumor properties of 3,5-bis(arylidene)-4-piperidones modified with organophosphorus groups is of significant interest for the design of new antiproliferative and anticancer agents.

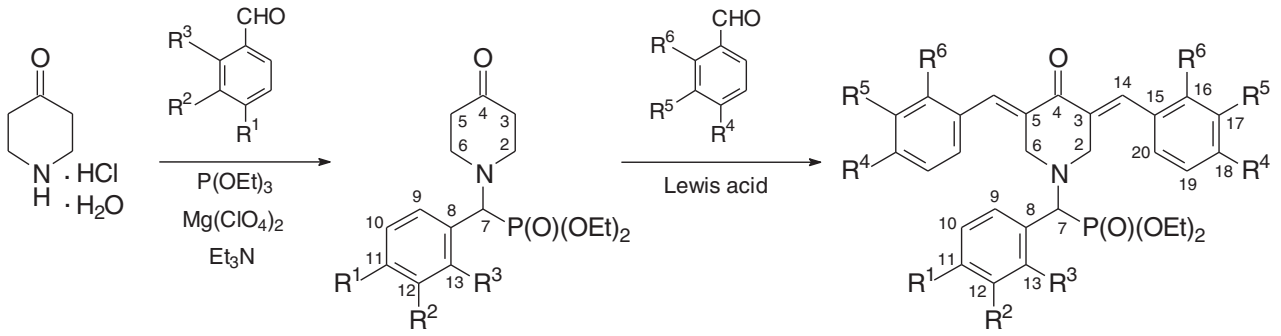
The aim of the this study is to develop a synthetic approach to novel 1,5-diaryl-3-oxo-1,4-pentadienes based on 4-piperidone modified with diethyl phosphonate group connected to the nitrogen atom of the piperidone scaffold through a short linking group. Recently we have elaborated a preparative procedure for the synthesis of diethyl α-amino (aryl)methyl phosphonates **1** (Scheme 1) containing 4-piperidone ring as an amine unit (Makarov et al. 2015). In the present work these diethyl [(4-oxopiperidin-1-yl)(aryl)methyl] phosphonates were used as starting compounds to prepare corresponding α-aminophosphonates of 3,5-bis(arylidene)-4-piperidone series **2**; the latter compounds were screened in vitro for inhibitory activity toward cancer cells.

Results and discussion

Synthesis and structure

Starting α-amino(aryl)methyl phosphonates **1a–e** required for the synthesis of the corresponding 3,5-bis(arylidene)-4-piperidones **2a–n** were obtained in moderate yields through Kabachnik-Fields reaction of 4-piperidone hydrochloride monohydrate, an aromatic aldehyde, and triethyl phosphite carried out in the presence of triethylamine and 25 mol % of magnesium perchlorate as illustrated in Scheme 1. Synthesis of compounds **1a–d** was reported previously (Makarov et al. 2015) while new phosphonate **1e** is described in the present article.

The next step of the proposed reaction sequence consisted in the crotonic condensation of phosphorylated 4-piperidones **1a–e** with aromatic aldehydes containing substituets differing in their electronic properties (from electron-withdrawing NO_2 and CN groups to electron-



1a ($R^1 = R^2 = R^3 = H$)
1b ($R^1 = F$, $R^2 = R^3 = H$)
1c ($R^1 = MeO$, $R^2 = R^3 = H$)
1d ($R^1 = R^2 = R^3 = MeO$)
1e ($R^1 = R^3 = MeO$, $R^2 = H$)

2	R^1	R^2	R^3	R^4	R^5	R^6
a	H	H	H	H	H	H
b	H	H	H	F	H	H
c	H	H	H	MeO	H	H
d	H	H	H	O_2N	H	H
e	F	H	H	F	H	H
f	F	H	H	MeO	H	H
g	F	H	H	Me_2N	H	H
h	F	H	H	MeO	MeO	MeO
i	F	H	H	O_2N	H	H
j	F	H	H	NC	H	H
k	MeO	H	H	H	H	H
l	MeO	H	MeO	F	H	H
m	MeO	MeO	MeO	MeO	MeO	MeO
n	F	H	H	H	OH	H

Scheme 1 Synthetic sequence used for the preparation of α-aminophosphonates of 3,5-bis(arylidene)-4-piperidone series **2a–n**

releasing MeO and Me_2N groups) to evaluate the effect of these properties on cytotoxicity of compounds. The crotonic condensation resulting in target compounds **2a–n** was performed in the presence of Lewis acidic systems: $LiClO_4$/Et_3N (system A) (Arnold et al. 2006) for condensations with benzaldehyde, 4-fluoro-, 4-methoxy-, 4-dimethylamino-, and 2,3,4-trimethoxybenzaldehyde or $BF_3{\cdot}OEt_2/CH_3CN$ (system B) (Leonova et al. 2010) for reactions with aldehydes containing strong electron-withdrawing substituents, such as 4-nitro- and 4-cyanobenzaldehyde, and with 3-hydroxybenzaldehyde. It should be pointed out that the application of standard acidic conditions (HCl in acetic acid) usually used for the synthesis of *N*-substituted 3,5-bis(arylidene)-4-piperidones was ruled out in our case because both starting substrates **1a–d** and condensation reaction products **2a–n** contain diethyl phosphonate group which is liable, in the presence of protonic acids, to partial hydrolysis into phosphonic acid group or may be even cleaved off the molecule under such conditions resulting in a number of by-products. Our experiments showed that $LiClO_4/Et_3N$ system gave unsatisfactory results in terms of yield and purity as regards preparing nitro, cyano and hydroxy derivatives **2d**, **i**, **j**, **n** (with this system we observed formation of mixtures of many products), in which case we used an acetonitrile solution of $BF_3{\cdot}OEt_2$ as a reaction medium providing better results. Furthermore, to ensure better mixing and, therefore, contact of starting reagents, which all are solids, synthesis of compounds **2g**, **h**, **m** was performed in the presence of higher amount of triethylamine (5 equivalents rather than 2 equivalents as in the case of compounds **2a–c**, **e**, **f**, **k**, **l**). Yields of 3,5-bis(arylidene)-4-piperidones **2a–n** varied in the range of 25–75 %, with the highest yield being obtained for compound **2m** and the lowest ones for nitro- and cyano-containing analogues **2d**, **i**, **j**.

Structures of compounds **2a–n** were confirmed by IR and multinuclear NMR spectroscopy. The IR spectra of **2a–n** show characteristic absorption bands at 1240–1255 (P=O),

1024–1032 (P–O–C), and 1668–1677 (C=O) cm^{-1}. The ^{31}P NMR spectra of these compounds contain one singlet at 21.5–22.5 ppm typical of diethyl phosphonate group. The ^{1}H NMR spectra have a characteristic doublet at ca. 4.05 ppm with a coupling constant $^{2}J_{PH} = 20$ Hz assigned to the single proton of the N-CH(Ar)-P unit. The carbon atom of this unit resonates in the ^{13}C NMR spectra as a doublet at ca. 65–66 ppm with a coupling constant $^{1}J_{CP} = 160$ Hz. The ^{19}F NMR spectra of fluorine-containing 3,5-bis(arylidene)-4-piperidones of series **2** contain singlets at ca. -111 (F-C^{18}) and/or ca. −114 (F-C^{11}) ppm depending on the structure. On the whole, the ^{1}H and ^{13}C NMR spectral data are consistent with *E,E*-configuration of vinyl bonds in 1,5-diaryl-3-oxo-1,4-pentadiene scaffold. Moreover, the molecular structure of fluorine-containing representative **2e** was unambiguously confirmed by X-ray diffraction analysis.

The main backbone of molecule **2e** (Fig. 2) contains three planar fragments; the first includes the basal plane of the piperidone cycle (PA), while the planar fragments PB and PC include a benzene ring and adjacent atoms. The dihedral angles PA/PB and PA/PC between these fragments are equal to 28.68(5)° and 26.61(5)°, respectively. The central piperidone cycle adopts a *sofa* conformation, with the deviation of the N1 nitrogen atom from the plane passed through the other atoms of the ring by 0.755(1) Å. The N1 nitrogen atom of the piperidone ring has a trigonal-pyramidal configuration (sum of bond angles at the N1 atom is 336.7(3)°).

The (phenyl)(methyl)phosphonate ligand occupies the more sterically favorable equatorial position. The rotation angle of the phosphonate ligand relative to the central piperidone ring, defined as the pseudo-torsion O2 = P1… N1–C2 angle, is equal to 171.7(1)°.

Phosphonates **2a–n** may be transformed into free phosphonic acids using treatment with bromotrimethylsilane followed by hydrolysis of intermediate silyl esters with aqueous methanol. As an illustrative example, possibility of such transformation is demonstrated for phosphonate **2e** (Scheme 2). The corresponding acid **3** was treated with aqueous ammonia in ethanol to give ammonium salt. Surprisingly, the elemental analysis showed that the ammonium salt formed under used conditions was a monoammonium salt **4**.

Acid **3** and salt **4** were characterized by the NMR spectroscopy data. The ^{31}P NMR spectra of these compounds contain a singlet at 16.7 ppm (compound **3**) and 13.3 ppm (compound **4**); their ^{19}F NMR spectra have two singlets at ca. -110.5 (F-C^{18}) and -116 (F-C^{11}) ppm corresponding to two different fluorine atoms, with the singlets intensity ratio being equal to 2:1. A characteristic doublet with $^{2}J_{PH} = 20$ Hz assigned to the proton of the CHP moiety (at 4.1 ppm for **3** and at 3.8 ppm for **4**) is observed in the ^{1}H NMR spectra of both compounds. Finally, the ^{13}C NMR spectra have a doublet at ca. 66 ppm with $^{1}J_{PC} = 140$ Hz (CHP moiety) and two doublets with $^{1}J_{CF} = 240$ in the range of 161–163 ppm due to C–F coupling. Other NMR signals are also in agreement with the structures drawn in Scheme 2.

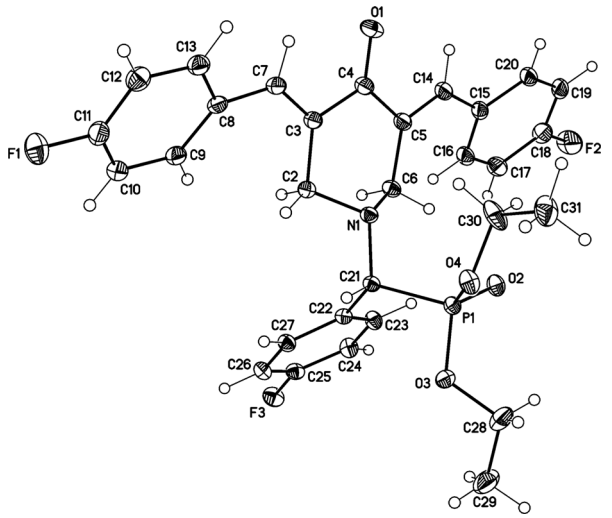
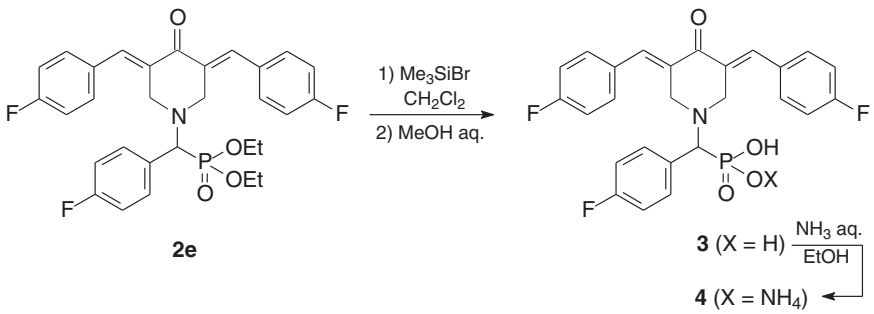


Fig. 2 Molecular structure of **2e** drawn at 40 % probability of anisotropic displacement ellipsoids. The alternative positions of the disordered ethyl groups are not shown

Scheme 2 Transformation of fluorine-containing phosphonate **2e** into corresponding phosphonic acid **3** and its ammonium salt **4**



Cytotoxic properties

The cytotoxic activity of the compounds **2a–n** was tested in vitro against human malignant cell lines, such as RD (rhabdomyosarcoma), PC3 (prostate cancer), HCT116 (colon cancer), MCF7 (breast cancer), and normal human embryonic kidney (HEK293) cell line. Our choice of these cell lines was based on the following considerations. First, according to the literature data various *N*-substituted 3,5-bis (arylidene)-4-piperidones demonstrated high in vitro cytotoxicity toward PC3, HCT116 and MCF7 cells (Thakur et al. 2014). Second, surprisingly only few publications describe data on inhibitory activity of dienone compounds toward malignant muscle tissue cells (Selvendiran et al. 2009). Finally, evaluation of cytotoxicity of compounds **2a–n** toward normal HEK293 cells was undertaken to ascertain whether these compounds have any selectivity for tumor cells.

The results obtained in this study are summarized in Table 1 showing the corresponding IC_{50} values (IC_{50} is the concentration of compound required to inhibit the growth of the cells by 50 %). Anticancer antibiotics Doxorubicin and Daunorubicin were used as positive controls. Moreover, for comparison purposes, we also evaluated antiproliferative properties of analogues **5** and **6** (Fig. 3) having structure similar to that of phosphonates **2a–n** except for aromatic ring attached to α-aminomethyl phosphonate residue.

In general, the majority of the studied phosphonates **2a–n** show IC_{50} values in the range of 2.5–8.5 μM, with the most evident exception being compound **2g** containing the dimethylamino groups in the bis(arylidene)piperidone framework. Such a low cytotoxicity of 3,5-bis(4-dimethylbenzylidene)-4-piperidones is known from other studies (Makarov et al. 2009) and may be attributed to a strong electron-releasing character of NMe_2 substituents. Compounds **2c**, **f**, **k**, **m** having methoxy substituents in the aromatic rings exhibit somewhat lower activity toward MCF7 cells as compared to other active compounds. The highest toxic activity toward the studied cancer cell lines is revealed by nitro-derivative **2d**, for which all inhibitory concentrations are lower than 3.0 μM. Another

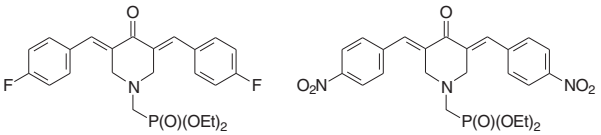


Fig. 3 Structures of fluorine (**5**) and nitro (**6**) analogues of compounds **2b** and **2d**

Table 1 Cytotoxicity of phosphonates **2a–n**, phosphonic acid **3** and salt **4** toward human malignant cell lines RD, PC3, HCT116 and MCF7, and normal HEK293 cells

Compound	IC_{50}, μM				
	RD	PC3	HCT116	MCF7	HEK293
2a	5.1 ± 0.1	6.5 ± 0.8	2.7 ± 0.1	4.0 ± 0.2	2.0 ± 0.5
2b	8.1 ± 0.2	6.8 ± 1.1	3.0 ± 1.4	3.8 ± 0.6	3.0 ± 1.0
2c	4.9 ± 0.1	7.8 ± 1.1	4.8 ± 0.3	12.0 ± 1.5	3.6 ± 0.5
2d	n/a[a]	2.2 ± 0.4	2.8 ± 0.5	2.4 ± 0.4	1.3 ± 0.3
2e	2.5 ± 0.1	7.2 ± 0.8	3.8 ± 0.8	6.0 ± 0.7	2.8 ± 0.6
2f	5.5 ± 0.1	8.0 ± 1.1	5.3 ± 0.1	12.2 ± 0.3	7.0 ± 0.7
2g	119 ± 20	n/a	191 ± 41	222 ± 30	n/a
2h	5.8 ± 0.1	6.4 ± 0.7	5.9 ± 0.1	8.5 ± 0.7	4.5 ± 0.6
2i	n/a	3.5 ± 0.5	n/a	3.0 ± 0.4	7.0 ± 0.6
2j	n/a	3.5 ± 0.6	3.8 ± 0.5	4.3 ± 0.6	3.5 ± 0.5
2k	n/a	4.6 ± 0.6	3.5 ± 0.4	12.0 ± 1.2	3.4 ± 0.4
2l	2.5 ± 0.1	6.2 ± 0.7	2.6 ± 0.1	6.5 ± 0.5	7.6 ± 0.7
2m	n/a	10.0 ± 0.8	7.0 ± 0.6	22.0 ± 2.0	8.5 ± 0.7
2n	n/a	7.5 ± 1.0	n/a	12.0 ± 1.5	21.0 ± 2.4
3	n/a	>100	n/a	>100	>100
4	n/a	>100	>100	>100	>100
5[b]	n/a	10.2 ± 0.7	5.5 ± 0.5	6.1 ± 0.5	3.6 ± 0.4
6[b]	n/a	9.1 ± 0.6	4.0 ± 0.5	6.5 ± 0.5	3.4 ± 0.3
Doxorubicin	0.53 ± 0.03	1.7 ± 0.2	0.19 ± 0.01	0.56 ± 0.03	0.24 ± 0.02
Daunorubicin	2.45 ± 0.07	n/a	0.21 ± 0.01	1.44 ± 0.31	n/a

[a] *n/a* not analyzed

[b] Compounds **5** and **6** were described previously (Makarov et al. 2009)

nitro-compound **2i** and nitrile analogue **2j**, also bearing strong electron-withdrawing substituents in benzylidene moieties, demonstrate good cytotoxicity toward cancer cell, as well (with IC_{50} values in the range of 3.0–4.3 μM). Although the results obtained do not allow us to draw a conclusion on any noticeable selectivity of the screened compounds toward a particular cell line, it may be noted that the lowest inhibitory concentrations (<3.0 μM) are shown by the following phosphonates: **2e**, **l** (toward RD cells), **2d** (toward PC3 cells), **2a**, **d**, **l** (toward HCT116 cells) and **2d**, **i** (toward MCF7 cells). On the whole, normal HEK293 cells are only slightly more sensitive to compounds **2** than cancer cells, with analog **2n** exhibiting even higher activity toward studied PC3 and MCF7 cell lines than toward normal cells. This fact justifies further investigations of the antiproliferative activity of phosphorylated 3,5-bis(arylidene)-4-piperidones with the aromatic rings bearing OH-substituents.

The comparison of the cytotoxic activity of phosphonates **2b** and **2d** with that of their corresponding analogues **5** and **6**, lacking phenyl ring at the methylene carbon atom connecting piperidone nitrogen atom and phosphonate group, indicates that the presence of this phenyl ring does not result in any adverse effect on the cytotoxicity. In contrast, compounds **2b** and **2d** proved to be even more potent than counterparts **5** and **6**. Compounds **2e**, **l** which also differ from analogue **5** only by the presence of aromatic ring at above mentioned methylene linker are not inferior to this analogue in terms of cytotoxic activity, as well.

We also evaluated the cytotoxic properties of phosphonic acid **3** and corresponding momoammonium salt **4** in the same 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test as used for related phosphonate **2e**. Surprisingly, both salt and acid turned out to be non-toxic ($IC_{50} > 100$ μM). This fact might be associated with inability of free acid and its salt to pass through cell membrane: for example, poor membrane permeability was mentioned as the major drawback of bisphosphonic acids (Ezra et al. 2000; Porras and Gertz 2004; Ledoux et al. 2006), with possible solution to overcome this problem being partial transformation of ionizable acidic phosphonic groups into corresponding esters.

Conclusion

This study demonstrates that modifying 3,5-bis(arylidene)-4-piperidone pharmacophore with phosphonate group attached to the piperidone nitrogen atom through (aryl)methylene unit results in novel antiproliferative agents with good inhibitory activity toward malignant cells: most of the resulting phosphonates are distinguished by IC_{50} values in the range of 2.5–8.0 μM. In general, the highest activity is revealed by 3,5-bis(arylidene)-4-piperidones containing electron-withdrawing substituents in the arylidene moieties. The comparison with the related phosphonates without aryl ring attached to methylene linker connecting piperidone scaffold and phosphonate group shows that such aryl ring does not interfere with the antitumor efficacy of compounds. Therefore, these diethyl (3,5-diarylidene-4-oxopiperidin-1-yl)(aryl) methylphosphonates **2** are of significant interest for further studies including an elucidation of the mechanistic aspects of their cytotoxic activity to determine a specific biological target thereof and reduce toxicity against normal cells along with an investigation of their in vivo effects.

Experimental

Chemistry

NMR spectra were recorded on a Bruker Avance 400 spectrometer (^{1}H, 400; ^{19}F, 376; ^{31}P, 162 and ^{13}C, 100 MHz) or Bruker Avance 300 spectrometer (^{1}H, 300; ^{19}F, 282; ^{31}P, 121 and ^{13}C, 75.5 MHz) using residual proton signal (^{1}H) and that of carbon atom (^{13}C) of a deuterated solvent as an internal standard relative to TMS and $CFCl_3$ (^{19}F), and H_3PO_4 (^{31}P) as an external standard. Column chromatography was carried out using Merck silica gel 60 (230–400 mesh ASTM). Analytical TLCs were performed with Merck silica gel 60 F254 plates. Visualization was accomplished by UV light. IR spectra were recorded in KBr pellets on a Magna-IR750 (Nicolet) Fourier spectrometer, resolution 2 cm^{-1}, 128 scans. Melting points were determined with a MPA 120 EZ-Melt Automated Melting Point Apparatus (USA) and were uncorrected. All commercial reagents were purchased from Acros and used without further purification. Synthesis of compounds **1a–d** was described previously (Makarov et al. 2015).

*Diethyl [(2,4-dimethoxyphenyl)(4-oxopiperidin-1-yl) methyl]phosphonate (**1e**)*

A mixture of piperidone hydrochloride monohydrate (0.77 g, 5 mmol), 2,4-dimethoxybenzaldehyde (0.83 g, 5 mmol), Et_3N (0.5 g, 5 mmol), anhydrous $Mg(ClO_4)_2$ (0.28 g, 1.25 mmol, 25 mol %), and $(EtO)_3P$ (0.83 g, 5 mmol) was stirred for 20 h at room temperature. Water and CH_2Cl_2 were added to the mixture, organic phase was separated, aqueous phase was extracted with CH_2Cl_2. Combined organic phase was washed with an aqueous Na_2CO_3 solution, separated, dried over Na_2SO_4, filtered and evaporated on a rotary evaporator to give crude product (2.0 g) as an oil containing 53 mol % of compound **1e**. The crude product was purified by column chromatography (column: *l*, 32 cm, *d*, 2 cm) using gradient elution starting with petroleum ether and continuing with petroleum

ether/acetone mixtures (gradient from 15:2 to 15:4). Evaporation of appropriate fractions on a rotary evaporator afforded the desired compound as yellowish oil (0.77 g, 40 %). ^{31}P NMR (162 MHz, $CDCl_3$): $\delta = 22.99$. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.05$ and 1.36 (both *t*, 6H, $^{3}J_{HH} = 7.1$ Hz, $POCH_2C\underline{H}_3$), 2.40 (*t*, 4H, $^{3}J_{HH} = 7.1$ Hz, $N(CH_2)_2$), 2.70–2.75 (m, 2H), 3.11–3.16 (m, 2H), 3.69–3.73–3.79 (m, 7H, $POCH_2$ + 2OMe), 3.87–3.97 (m, 1H, $POCH_2$), 4.21–4.30 (m, 2H, $POCH_2$), 4.71 (d, 1H, $^{2}J_{PH} = 24$ Hz, PCH), 6.45 (s, 1H, C_6H_3), 6.48 (d, 1H, $^{3}J_{HH} = 8$ Hz, C_6H_3), 7.68 (d, 1H, $^{3}J_{HH} = 8$ Hz, C_6H_3). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.2$ and 16.6 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 41.9 (C^3, C^5), 51.1 (d, $^{3}J_{PC} = 9$ Hz, C^2, C^6), 55.3 (OMe), 55.5 (OMe), 56.7 (d, $^{1}J_{PC} = 166$ Hz, C^7), 62.2 and 63.0 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 98.4 (C^{12}), 103.9 (C^{10}), 112.7 (d, $^{2}J_{PC} = 5$ Hz, C^8), 132.0 (d, $^{3}J_{PC} = 4$ Hz, C^9), 159.0 (d, $^{3}J_{PC} = 12$ Hz, C^{13}), 160.7 (C^{11}), 209.0 (C^4). Found (%): C, 56.19; H, 7.45; N, 3.68. Calc. for $C_{18}H_{28}NO_6P$ (%): C, 56.10; H, 7.32; N, 3.63.

General procedure A for the synthesis of compounds ***2a–c, e–h, k–m***

Diethyl (4-oxopiperidin-1-yl)(aryl)methylphosphonate (1.5 mmol) was mixed with an aromatic aldehyde (3.0 mmol) and triethylamine (0.30 g, 3.0 mmol in the case of compounds **2a–c**, **e**, **f**, **k**, **l**; 0.75 g, 7.5 mmol in the case of compounds **2g**, **h**, **m**). Lithium perchlorate (0.32 g, 3.0 mmol) was added to the stirred mixture and stirring was continued for 48 h at room temperature. Then, water and CH_2Cl_2 were added to the reaction mixture. Organic layer was separated, washed with water, dried over Na_2SO_4, filtered through filter paper, and evaporated to dryness on a rotary evaporator to afford crude product that was subjected to SiO_2 column chromatography (column: *l*, 30 cm, *d*, 2 cm). Details for elution conditions are given below for each compound individually. Appropriate fractions obtained from a column were evaporated on a rotary evaporator to give desired compounds.

General procedure B for the synthesis of compounds ***2d, i, j***

Diethyl (4-oxopiperidin-1-yl)(aryl)methylphosphonate (1.5 mmol) was mixed with an aromatic aldehyde (3.0 mmol) in anhydrous acetonitrile (2 mL). Boron trifluoride etherate (1.28 g, 9.0 mmol) was added to the stirred mixture and stirring was continued for 48 h at room temperature. Then, Et_2O (25 mL) was added to the reaction mixture and the solid formed was filtered off. The solid obtained was added to a solution of sodium carbonate (0.9 g) in water (20 mL) and the mixture was stirred for 2 h. Organic substance was filtered off and dried in air to afford crude product that was subjected to SiO_2 column chromatography (column: *l*, 22 cm, *d*, 2 cm). Elution was started with CH_2Cl_2 and continued with a 150:5 CH_2Cl_2/EtOH mixture and 100:5 CH_2Cl_2/EtOH mixture. Appropriate fractions obtained from a column were evaporated on a rotary evaporator to give desired compounds.

*Diethyl (3,5-dibenzylidene-4-oxopiperidin-1-yl)(phenyl) methylphosphonate (**2a**)*

Column chromatography: elution was started with a 10:1 petroleum ether/acetone mixture and continued with 5:1 petroleum ether/acetone mixture. Evaporation of eluates gave yellow crystalline product (0.44 g, 58 %), m.p. 137–142 °C. IR (KBr), ν_{max}, cm^{-1}: 1670 (C=O), 1609 (C=C), 1581 (C=C), 1570 (C=C), 1224 (P=O), 1022 (P–O). ^{31}P NMR ($CDCl_3$, 162 MHz): $\delta = 22.02$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.97$ and 1.10 (both *t*, 6H, $^{3}J_{HH} = 7.0$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.57–3.65 (m, 1H, $POCH_2$), 3.82–4.02 (m, 5H, CH_2 (cyclic) + $POCH_2$), 4.09 (d, 1H, CHP, $^{2}J_{PH} = 20.0$ Hz), 4.27–4.31 (m, 2H, CH_2 (cyclic)), 7.31–7.49 (m, 15H, $C_{Ph}H$), 7.78 (s, 2H, $C^{14}H$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.1$ and 16.3 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 53.0 (d, $^{3}J_{PC} = 8$ Hz, C^2, C^6), 62.5 and 62.8 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 66.4 (d, $^{1}J_{PC} = 159$ Hz, C^7), 128.3 (C^8), 128.42 (C^{10}, C^{12}), 128.6 (C^{17}, C^{19}), 129.0 (C^{18}), 130.2 (d, $^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 130.4 (C^{16}, C^{20}), 132.4 (C^{11}), 133.3 (C^{15}), 135.1 (C^3, C^5), 136.5 (C^{14}), 187.6 (C^4). Anal. Calcd. for $C_{30}H_{32}NO_4P$ (%): C, 71.84; H, 6.43; N, 2.79. Found (%): C, 71.74; H, 6.57; N, 2.75.

*Diethyl (3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl) (phenyl)methylphosphonate (**2b**)*

Column chromatography: elution was started with a 4:1 $CHCl_3$/hexane mixture and continued with a 100:1 $CHCl_3$/EtOH mixture. Evaporation of eluates gave yellow crystalline product (0.38 g, 47 %), m.p. 129–131 °C. IR (KBr), ν_{max}, cm^{-1}: 1676 (C=O), 1616 (C=C), 1600 (C=C), 1582 (C=C), 1509 (C=C), 1226 (P=O), 1026 (P–O). ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 21.84$. ^{19}F NMR ($CDCl_3$, 376 MHz): $\delta = -111.33$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.96$ and 1.10 (both *t*, 6H, $^{3}J_{HH} = 7.1$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.53–3.63 (m, 1H, $POCH_2$), 3.79–4.00 (m, 5H, CH_2 (cyclic) + $POCH_2$), 4.05 (d, 1H, $^{2}J_{PH} = 20.0$ Hz, CHP), 4.22–4.25 (m, 2H, CH_2 (cyclic)), 7.07 (*t*, 4H, $^{3}J_{HH} = {}^{3}J_{HF} = 8.6$ Hz, $C^{17}H$ and $C^{19}H$), 7.25–7.33 (m, 7H, $C_{Ph}H + C^{16}H$ and $C^{20}H$), 7.45 (m, 2H, $C_{Ph}H$), 7.71 (s, 2H, $C^{14}H$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.1$ and 16.3 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 52.8 (d, $^{3}J_{PC} = 9$ Hz, C^2, C^6), 62.6 and 62.8 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 66.4 (d, $^{1}J_{PC} = 161$ Hz, C^7), 115.7 (d, $^{2}J_{FC} = 21$ Hz, C^{17}, C^{19}), 128.4 (C^8), 128.5 (C^{10}, C^{12}), 130.1 (d, $^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 131.3 (d, $^{4}J_{CF} = 3$ Hz, C^{15}), 132.3 (d, $^{3}J_{CF} = 9$ Hz, C^{16}, C^{20}), 132.4 (C^{11}), 132.9 (C^3, C^5), 135.4

(C^{14}), 162.9 (d, $^{1}J_{CF} = 250$ Hz, C^{18}), 187.3 (C^{4}). Anal. Calcd. for $C_{31}H_{30}F_2NO_4P$ (%): C, 67.03; H, 5.63; N, 2.61. Found (%): C, 66.91; H, 5.80; N, 2.51.

*Diethyl (3,5-bis(4-methoxybenzylidene)-4-oxopiperidin-1-yl)(phenyl)methylphosphonate (**2c**)*

Column chromatography: elution was started with petroleum ether and continued with petroleum ether/acetone mixtures with gradient from 10:1.5 to 10:2 and 10:2.5. Evaporation of eluates gave yellow crystalline product (0.55 g, 67 %), m.p. 125–126 °C. IR (KBr), ν_{max}, cm^{-1}: 1672 (C=O), 1599 (C=C), 1510 (C=C), 1253 (P=O), 1019 (P–O). ^{31}P NMR ($CDCl_3$, 162 MHz): $\delta = 21.85$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.97$ and 1.10 (both *t*, 6H, $^{3}J_{HH} = 7.0$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.59–3.65 (m, 1H, $POCH_2$), 3.82 (s, 6H, OCH_3), 3.84–4.01 (m, 5H, CH_2 (cyclic) + $POCH_2$), 4.08 (d, 1H, CHP, $^{2}J_{PH} = 20.7$ Hz), 4.24–4.28 (m, 2H, CH_2 (cyclic)), 6.90 (d, 4H, $^{3}J_{HH} = 8.9$ Hz), 7.29–7.32 (m, 7H), 7.50 (d, 2H, $^{3}J_{HH} = 7.0$ Hz), 7.70 (s, 2H, C^{14}H). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.1$ and 16.3 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 53.0 (d, $^{3}J_{PC} = 9$ Hz, C^2, C^6), 55.3 (OMe), 62.4 and 62.9 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 66.6 (d, $^{1}J_{PC} = 161$ Hz, C^7), 114.1 (C^{17}, C^{19}), 127.9 (C^8), 128.30 (C^{15}), 128.4 (C^{10}, C^{12}), 130.2 (d, $^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 131.5 (C^3, C^5), 132.3 (C^{16}, C^{20}), 132.4 (C^{11}), 135.9 (C^{14}), 160.2 (C^{18}), 187.4 (C^4). Anal. Calcd. for $C_{32}H_{36}NO_6P$ (%): C, 68.44; H, 6.46; N, 2.49. Found (%): C, 68.47; H, 6.54; N, 2.47.

*Diethyl (3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl)(phenyl)methylphosphonate (**2d**)*

Product obtained after column chromatography was additionally purified by precipitation with hexane from its solution in ethyl acetate to give yellow powder in a yield of 0.26 g (29 %), m.p. 175–180 °C. IR (KBr), ν_{max}, cm^{-1}: 1671 (C=O), 1616 (C=C), 1597 (C=C), 1523 (NO_2), 1346 (NO_2), 1246 (P=O), 1021 (P–O). ^{31}P NMR ($CDCl_3$, 162 MHz): $\delta = 21.02$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.97$ and 1.14 (both *t*, 6H, $^{3}J_{HH} = 7.1$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.55–3.65 (m, 1H, $POCH_2$), 3.83–4.05 (m, 6H, $POCH_2$ + CH_2 (cyclic) + CHP), 4.27–4.31 (m, 2H, CH_2 (cyclic)), 7.30–7.35 (m, 3H), 7.43–7.45 (m, 2H), 7.47 (d, 4H, $^{3}J_{HH} = 8$ Hz), 7.76 (s, 2H, C^{14}H), 8.25 (d, 4H, $^{3}J_{HH} = 8$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.1$ and 16.4 (both d, $^{3}J_{PC} = 5$ Hz, $POCH_2\underline{C}H_3$), 52.7 (d, $^{3}J_{PC} = 8$ Hz, C^2, C^6), 62.8 and 62.9 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 66.4 (d, $^{1}J_{PC} = 160$ Hz, C^7), 123.8 (C^{17}, C^{19}), 128.7 (C^8), 128.7 (C^{10}, C^{12}), 130.1 (d, $^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 130.8 (C^{16}, C^{20}), 132.0 (C^{11}), 134.2 (C^3, C^5), 135.9 (C^{14}), 141.2 (C^{15}), 147.4 (C^{18}–NO_2), 162.6 (d, $^{1}J_{CF} = 247$ Hz, C^{11}), 186.3 (C^4). Anal. Calcd. for $C_{30}H_{30}N_3O_8P$ (%): C, 60.91; H, 5.11; N, 7.10. Found (%): 60.87; H, 5.18; N, 7.19.

*Diethyl (3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl)(4-fluorophenyl)methylphosphonate (**2e**)*

Column chromatography: elution was started with a 10:1 petroleum ether/acetone mixture with gradient to 10:2 petroleum ether/acetone and 10:3 petroleum ether/acetone mixtures. Evaporation of eluates gave yellow crystalline product (0.52 g, 63 %), m.p. 145–150 °C. IR (KBr), ν_{max}, cm^{-1}: 1673 (C=O), 1614 (C=C), 1600 (C=C), 1578 (C=C), 1511 (C=C), 1506 (C=C), 1231 (P=O), 1026 (P–O). ^{31}P NMR ($CDCl_3$, 162 MHz): $\delta = 21.52$. ^{19}F NMR ($CDCl_3$, 376 MHz): $\delta = -110.58$ (F-C^{18}), -113.12 (F-C^{11}). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.98$ and 1.10 (both *t*, 6H, $^{3}J_{HH} = 7.0$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.57–3.66 (m, 1H, $POCH_2$), 3.79–3.99 (m, 5H, CH_2 (cyclic) + $POCH_2$), 4.02 (d, 1H, $^{2}J_{PH} = 20.0$ Hz, CHP), 4.18–4.22 (m, 2H, CH_2 (cyclic)), 6.97 (*t*, 2H, $^{3}J_{HH} = {}^{3}J_{HF} = 8.6$ Hz, C^{10}H and C^{12}H), 7.07 (*t*, 4H, $^{3}J_{HH} = {}^{3}J_{HF} = 8.7$ Hz, C^{17}H and C^{19}H), 7.30 (dd, 4H, $^{3}J_{HH} = 8.6$ Hz, $^{4}J_{HF} = 5.4$ Hz, C^{16}H and C^{20}H), 7.42 (dd, 2H, $^{3}J_{HH} = 7.3$ Hz, $^{4}J_{HF} = 5.4$ Hz, C^9H and C^{13}H), 7.70 (s, 2H, C^{14}H). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.1$ and 16.3 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 52.7 (d, $^{3}J_{PC} = 9$ Hz, C^2, C^6), 62.6 and 62.9 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 66.3 (d, $^{1}J_{PC} = 160$ Hz, C^7), 115.4 (d, $^{2}J_{FC} = 21$ Hz, C^{10}, C^{12}), 115.8 (d, $^{2}J_{FC} = 22$ Hz, C^{17}, C^{19}), 128.5 (d, $^{4}J_{FC} = 3$ Hz, C^8), 131.2 (d, $^{4}J_{CF} = 4$ Hz, C^{15}), 131.7 (*t*, $^{3}J_{FC} = {}^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 132.3 (d, $^{3}J_{CF} = 8$ Hz, C^{16}, C^{20}), 132.7 (C^3, C^5), 135.6 (C^{14}), 162.6 (d, $^{1}J_{CF} = 248$ Hz, C^{11}), 162.9 (d, $^{1}J_{CF} = 251$ Hz, C^{18}), 187.1 (C^4). Anal. Calcd. for $C_{30}H_{29}F_3NO_4P$ (%): C, 64.86; H, 5.26; N, 2.52. Found (%): C, 64.83; H, 5.36; N, 2.48.

*Diethyl (3,5-bis(4-methoxybenzylidene)-4-oxopiperidin-1-yl)(4-fluorophenyl)methylphosphonate (**2f**)*

Column chromatography: elution was started with petroleum ether and continued with petroleum ether/acetone mixtures with gradient from 10:1 to 10:2 and 10:2.5. Evaporation of eluates gave yellow viscous product that was dissolved in CH_2Cl_2 (2.5 ml) and precipitated with pentane in freezer (-20 °C) to give yellow crystalline product (0.29 g, 33 %), m.p. 140–152 °C. IR (KBr), ν_{max}, cm^{-1}: 1665 (C=O), 1599 (C=C), 1564 (C=C), 1511 (C=C), 1256 (P=O), 1028 (P–O). ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 21.77$. ^{19}F NMR ($CDCl_3$, 376 MHz): $\delta = -113.56$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.98$ and 1.09 (both *t*, 6H, $^{3}J_{HH} = 7.0$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.59–3.65 (m, 1H, $POCH_2$), 3.82 (s, 6H, OCH_3), 3.86–4.00 (m, 5H, CH_2 (cyclic) + $POCH_2$), 4.06 (d, 1H, CHP, $^{2}J_{PH} = 20.0$ Hz), 4.21–4.25 (m, 2H, CH_2 (cyclic)), 6.90 (d, 4H, $^{3}J_{HH} = 8.6$ Hz), 6.97 (*t*, 2H, $^{3}J_{HH} = {}^{3}J_{HF} = 8.7$ Hz, C^{10}H and C^{12}H), 7.28 (d, 4H, $^{3}J_{HH} = 8.6$ Hz), 7.45 (virtual *t*, 2H, $J = 6.5$ Hz, C^9H and C^{13}H), 7.71 (s, 2H, C^{14}H). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.1$ and 16.3

(both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 52.8 (d, $^{3}J_{PC} = 8$ Hz, C^2, C^6), 55.3 (OMe), 62.5 and 62.9 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 65.4 (d, $^{1}J_{PC} = 160$ Hz, C^7), 114.1 (C^{17}, C^{19}), 115.3 (d, $^{2}J_{FC} = 21$ Hz, C^{10}, C^{12}), 127.8 (C^{15}), 128.6 (d, $^{4}J_{FC} = 2$ Hz, C^8), 131.2 (C^3, C^5), 131.8 (*t*, $^{3}J_{FC} = {}^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 132.2 (C^{16}, C^{20}), 136.2 (C^{14}), 160.3 (C^{18}), 162.6 (d, $^{1}J_{CF} = 245$ Hz, C^{11}), 187.3 (C^4). Anal. Calcd. for $C_{32}H_{35}FNO_6P$ (%): C, 66.31; H, 6.09; N, 2.42. Found (%): C, 66.30; H, 6.17; N, 2.41.

*Diethyl (3,5-bis(4-dimethylaminobenzylidene)-4-oxopiperidin-1-yl)(4-fluorophenyl)methylphosphonate (**2g**)*

Column chromatography: elution was started with a 3:1 petroleum ether/acetone mixture, continued with a 2.5:1 petroleum ether/acetone mixture and finished with a 2:1 petroleum ether/acetone mixture. Evaporation of eluates gave red powder product (0.56 g) purified by slow diffusion precipitation with petroleum ether from a solution in acetone to give red crystalline product (0.32 g, 34 %), m.p. 178–184 °C. IR (KBr), ν_{max}, cm^{-1}: 1663 (C=O), 1588 (C=C), 1524 (C=C), 1231 (P=O), 1022 (P–O). ^{31}P NMR ($CDCl_3$, 162 MHz): $\delta = 21.96$. ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -113.97$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.01$ and 1.11 (both *t*, 6H, $^{3}J_{HH} = 7.0$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.01 (s, 12H, Me_2N), 3.63–3.72 (m, 1H, $POCH_2$), 3.85–4.03 (m, 5H, $POCH_2 + CH_2$ (cyclic)), 4.11 (d, 1H, CHP, $^{2}J_{PH} = 20.4$ Hz), 4.24–4.27 (m, 2H, CH_2 (cyclic)), 6.68 (d, 4H, $^{3}J_{HH} = 8.5$ Hz), 6.99 (*t*, 2H, $^{3}J_{HH} = {}^{3}J_{HF} = 8.0$ Hz, $C^{10}H$ and $C^{12}H$), 7.26 (d, 4H, $^{3}J_{HH} = 8.5$ Hz), 7.50 (*t*, 2H, $^{4}J_{HF} = 6.0$ Hz, C^9H and $C^{13}H$), 7.70 (s, 2H, $C^{14}H$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.2$ and 16.3 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 40.1 (Me_2N), 53.1 (d, $^{3}J_{PC} = 9$ Hz, C^2, C^6), 62.4 and 63.1 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 65.6 (d, $^{1}J_{PC} = 160$ Hz, C^7), 111.8 (C^{17}, C^{19}), 115.2 (d, $^{2}J_{FC} = 21$ Hz, C^{10}, C^{12}), 123.3 (C^{15}), 128.8 (C^8), 129.2 (C^3, C^5), 131.9 (*t*, $^{3}J_{FC} = {}^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 132.5 (C^{16}, C^{20}), 136.6 (C^{14}), 150.6 (C^{18}), 162.6 (d, $^{1}J_{CF} = 246$ Hz, C^{17}), 187.2 (C^4). Anal. Calcd. for $C_{34}H_{41}FN_3O_4P$ (%): C, 67.42; H, 6.82; N, 6.94. Found (%): C, 67.31; H, 6.85; N, 6.91.

*Diethyl (3,5-bis(2,3,4-trimethoxybenzylidene)-4-oxopiperidin-1-yl)(4-fluorophenyl)methylphosphonate (**2h**)*

Column chromatography: elution was carried out with a 5:1 petroleum ether/acetone mixture, continued with a 3:1 petroleum ether/acetone mixture and finished with a 2.5:1 petroleum ether/acetone mixture. Evaporation of eluates gave yellow powder product (0.64 g) purified by slow diffusion precipitation with petroleum ether from solution in acetone to give yellow crystalline product (0.40 g, 38 %), m.p. 130–134 °C. IR (KBr), ν_{max}, cm^{-1}: 1666 (C=O), 1605 (C=C), 1593 (C=C), 1572 (C=C), 1214 (P=O), 1028 (P–O). ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 22.07$. ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -113.75$. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 0.96$ and 1.07 (both *t*, 6H, $^{3}J_{HH} = 7.0$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.52–3.64 (m, 1H, $POCH_2$), 3.82–3.95 (m, 23H, 6 $OCH_3 + CH_2$ (cyclic) + $POCH_2$), 4.03 (d, 1H, CHP, $^{2}J_{PH} = 19.7$ Hz), 4.13–4.18 (m, 2H, CH_2 (cyclic)), 6.64 (d, 2H, $^{3}J_{HH} = 8.7$ Hz), 6.85 (d, 2H, $^{3}J_{HH} = 8.7$ Hz), 6.93 (*t*, 2H, $^{3}J_{HH} = {}^{3}J_{HF} = 8.6$ Hz, $C^{10}H$ and $C^{12}H$), 7.40 (dd, 2H, $^{3}J_{HH} = 7.2$ Hz, $^{4}J_{HF} = 5.6$ Hz, C^9H and $C^{13}H$), 7.96 (s, 2H, $C^{14}H$). ^{13}C NMR ($CDCl_3$, 75.5 MHz): $\delta = 16.1$ and 16.2 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 52.9 (d, $^{3}J_{PC} = 9$ Hz, C^2, C^6), 56.0 (OMe), 60.9 (OMe), 61.5 (OMe), 62.5 and 62.9 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 64.5 (d, $^{1}J_{PC} = 161$ Hz, C^7), 106.8 (C^{19}), 115.2 (d, $^{2}J_{FC} = 21$ Hz, C^{10}, C^{12}), 122.2 (C^{15}), 125.1 (C^{20}), 128.7 (C^8), 131.8 (*t*, $^{3}J_{FC} = {}^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 132.1 (C^3, C^5 overlapped with C^{14}), 142.2 (C^{17}), 153.5 (C^{18}), 154.8 (C^{16}), 162.5 (d, $^{1}J_{CF} = 246$ Hz, C^{11}), 187.1 (C^4). Anal. Calcd. for $C_{36}H_{43}FNO_{10}P$ (%): C, 61.80; H, 6.19; N, 2.00. Found (%): C, 61.94; H, 6.19; N, 2.02.

*Diethyl (3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl)(4-fluorophenyl)methylphosphonate (**2i**)*

Product obtained after column chromatography was additionally purified by precipitation with petroleum ether from its acetone solution to give yellow powder in a yield of 0.24 g (25 %), m.p. 184–186 °C. IR (KBr), ν_{max}, cm^{-1}: 1670 (C=O), 1615 (C=C), 1594 (C=C), 1523 (NO_2), 1346 (NO_2), 1245 (P=O), 1020 (P–O). ^{31}P NMR ($CDCl_3$, 162 MHz): $\delta = 20.69$. ^{19}F NMR ($CDCl_3$, 376 MHz): $\delta = -112.31$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.00$ and 1.15 (both *t*, 6H, $^{3}J_{HH} = 7.1$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.61–3.71 (m, 1H, $POCH_2$), 3.81–3.92 (m, 3H, $POCH_2 + CH_2$ (cyclic)), 3.94–4.04 (m, 3H, CHP, $^{2}J_{PH} = 20.5$ Hz, + $POCH_2$), 4.25–4.29 (m, 2H, CH_2 (cyclic)), 7.02 (*t*, 2H, $^{3}J_{HH} = {}^{3}J_{HF} = 8.0$ Hz, $C^{10}H$ and $C^{12}H$), 7.42 (dd, 2H, $^{3}J_{HH} = 7.7$ Hz, $^{4}J_{HF} = 5.6$ Hz, C^9H and $C^{13}H$), 7.48 (d, 4H, $^{3}J_{HH} = 8.5$ Hz), 7.76 (s, 2H, $C^{14}H$), 8.26 (d, 4H, $^{3}J_{HH} = 8.6$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.0$ and 16.2 (both d, $^{3}J_{PC} = 5$ Hz, $POCH_2\underline{C}H_3$), 52.5 (d, $^{3}J_{PC} = 8$ Hz, C^2, C^6), 62.6 and 62.9 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 65.3 (d, $^{1}J_{PC} = 159$ Hz, C^7), 115.5 (d, $^{2}J_{FC} = 21$ Hz, C^{10}, C^{12}), 123.7 (C^{17}, C^{19}), 127.8 (d, $^{2}J_{PC} = 3$ Hz, C^8), 130.6 (C^{16}, C^{20}), 131.6 (*t*, $^{3}J_{FC} = {}^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 134.0 (C^3, C^5), 135.6 (C^{14}), 141.0 (C^{15}), 147.4 (C^{18}), 162.6 (d, $^{1}J_{CF} = 247$ Hz, C^{11}), 186.3 (C^4). Anal. Calcd. for $C_{30}H_{29}FN_3O_8P$ (%): C, 59.11; H, 4.80; N, 6.89. Found (%): C, 59.18; H, 4.88; N, 6.98.

*Diethyl (3,5-bis(4-cyanobenzylidene)-4-oxopiperidin-1-yl)(4-fluorophenyl)methylphosphonate (**2j**)*

Product obtained after column chromatography was additionally purified by precipitation with petroleum ether from

its dichloromethane solution to give yellow powder in a yield of 0.32 g (37 %), m.p. 156–163 °C. IR (KBr), ν_{max}, cm^{-1}: 2228 (CN), 1671 (C=O), 1616 (C=C), 1604 (C=C), 1509 (C=C), 1247 (P=O), 1018 (P–O). ^{31}P NMR ($CDCl_3$, 162 MHz): $\delta = 20.98$. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -112.48. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.99$ and 1.13 (both *t*, 6H, $^{3}J_{HH} = 7.1$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.58–3.68 (m, 1H, $POCH_2$), 3.80–4.02 (m, 6H, $POCH_2 + CH_2$ (cyclic) + CHP), 4.20–4.24 (m, 2H, CH_2 (cyclic)), 7.00 (*t*, 2H, $^{3}J_{HH} = {}^{3}J_{HF} = 8.4$ Hz, $C^{10}H$ and $C^{12}H$), 7.39–7.41 (m, 6H), 7.68 (d, 4H, $^{3}J_{HH} = 8.2$ Hz), 7.70 (s, 2H, $C^{14}H$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.1$ and 16.4 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 52.6 (d, $^{3}J_{PC} = 8$ Hz, C^2, C^6), 62.7 and 63.0 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 65.4 (d, $^{1}J_{PC} = 159$ Hz, C^7), 112.5 (C^{18}), 115.6 (d, $^{2}J_{FC} = 22$ Hz, C^{10}, C^{12}), 118.3 (CN), 128.1 (d, $^{2}J_{PC} = 4$ Hz, C^8), 130.5 (C^{16}, C^{20}), 131.7 (*t*, $^{3}J_{FC} = {}^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 132.3 (C^{17}, C^{19}), 134.6 (C^3, C^5), 135.4 (C^{14}), 139.3 (C^{15}), 162.7 (d, $^{1}J_{CF} = 247$ Hz, C^{11}), 186.5 (C^4). Anal. Calcd. for $C_{32}H_{29}FN_3O_4P$ (%): C, 67.48; H, 5.13; N, 7.38. Found (%): C, 67.37; H, 5.18; N, 7.29.

*Diethyl (3,5-dibenzylidene-4-oxopiperidin-1-yl)(4-methoxyphenyl)methylphosphonate (**2k**)*

Column chromatography: elution was started with a 10:1 petroleum ether/acetone mixture with a gradient to 10:2 petroleum ether/acetone and 10:3 petroleum ether/acetone mixtures. Evaporation of eluates gave yellow viscous substance (0.51 g, 68 %) that was dissolved in Et_2O and evaporated on an aspirator to give foam that was triturated to yellow powder, m.p. 50–55 °C. IR (KBr), ν_{max}, cm^{-1}: 1672 (C=O), 1611 (C=C), 1584 (C=C), 1511 (C=C), 1253 (P=O), 1028 (P–O). ^{31}P NMR ($CDCl_3$, 162 MHz): $\delta = 22.08$. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.97$ and 1.08 (both *t*, 6H, $^{3}J_{HH} = 7.0$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.57–3.60 (m, 1H, $POCH_2$), 3.77, (s, 3H, OCH_3), 3.80–3.96 (m, 5H, CH_2 (cyclic) + $POCH_2$), 4.01 (d, 1H, CHP, $^{2}J_{PH} = 20.0$ Hz), 4.26–4.29 (m, 2H, CH_2 (cyclic)), 6.80 (d, 2H, $^{3}J_{HH} = 8.0$ Hz), 7.32–7.40 (m, 12H, $C_{Ar}H$), 7.77 (s, 2H, $C^{14}H$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.2$ and 16.3 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 52.8 (d, $^{3}J_{PC} = 9$ Hz, C^2, C^6), 55.2 (OMe), 62.5 and 62.8 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 65.6 (d, $^{1}J_{PC} = 160$ Hz, C^7), 113.8 (C^{10}, C^{12}), 124.2 (C^8), 128.5 (C^{17}, C^{19}), 129.0 (C^{18}), 130.4 (C^{16}, C^{20}), 131.4 (d, $^{3}J_{PC} = 8$ Hz, C^9, C^{13}), 133.4 (C^{15}), 135.2 (C^3, C^5), 136.5 (C^{14}), 159.6 (C^{11}), 187.6 (C^4). Anal. Calcd. for $C_{31}H_{34}NO_5P$ (%): C, 70.04; H, 6.45; N, 2.63. Found (%): C, 69.97; H, 6.59; N, 2.71.

*Diethyl (3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl)(2,4-dimethoxyphenyl)methylphosphonate (**2l**)*

Column chromatography: elution was carried out with a 5:1 petroleum ether/acetone mixture. Evaporation of eluates gave yellow crystalline product (0.42 g, 47 %), m.p. 151–155 °C. IR (KBr), ν_{max}, cm^{-1}: 1674 (C=O), 1614 (C=C), 1600 (C=C), 1582 (C=C), 1509 (C=C), 1233 (P=O), 1030 (P–O). ^{31}P NMR ($CDCl_3$, 162 MHz): δ = 22.53. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -110.99. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.00$ and 1.13 (both *t*, 6H, $^{3}J_{HH} = 7.1$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.65–3.93 (m, 10H, $POCH_2 + OCH_3 + CH_2$ (cyclic)), 4.00–4.05 (m, 2H, $POCH_2$), 4.21–4.25 (m, 2H, CH_2 (cyclic)), 4.74 (d, 1H, CHP, $^{2}J_{PH} = 24.0$ Hz), 6.48–6.52 (m, 2H), 7.09 (*t*, 4H, $^{3}J_{HH} = {}^{3}J_{HF} = 8.7$ Hz, $C^{17}H$ and $C^{19}H$), 7.36 (dd, 4H, $^{3}J_{HH} = 8.4$ Hz, $^{4}J_{HF} = 5.4$ Hz, $C^{16}H$ and $C^{20}H$), 7.64 (s, 2H, $C^{14}H$), 7.77 (d, 1H, $^{3}J_{HH} = 8.5$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.2$ and 16.4 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 52.8 (d, $^{3}J_{PC} = 9$ Hz, C^2, C^6), 55.3 (OMe), 55.4 (OMe), 56.6 (d, $^{1}J_{PC} = 167$ Hz, C^7), 62.2 and 63.2 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 98.4 (C^{12}), 104.1 (C^{10}), 111.8 (d, $^{2}J_{PC} = 5$ Hz, C^8), 115.6 (d, $^{2}J_{FC} = 21$ Hz, C^{17}, C^{19}), 131.4 (d, $^{4}J_{CF} = 3$ Hz, C^{15}), 132.0 (d, $^{3}J_{CP} = 4$ Hz, C^9), 132.4 (d, $^{3}J_{CF} = 8$ Hz, C^{16}, C^{20}), 133.6 (C^3, C^5), 134.5 (C^{14}), 159.1 (d, $^{3}J_{PC} = 11$ Hz, C^{13}), 160.9 (C^{11}), 162.8 (d, $^{1}J_{CF} = 250$ Hz, C^{18}), 187.3 (C^4). Anal. Calcd. for $C_{32}H_{34}F_2NO_6P$ (%): C, 64.32; H, 5.73; N, 2.34. Found (%): C, 64.11; H, 5.64; N, 2.43.

*Diethyl (3,5-bis(2,3,4-trimethoxybenzylidene)-4-oxopiperidin-1-yl)(2,3,4-trimethoxyphenyl) methylphosphonate (**2m**)*

Column chromatography: elution was started with CH_2Cl_2 and continued with a 10:1 CH_2Cl_2/acetone mixture. Evaporation of eluates gave yellow crystalline product (0.85 g, 73 %), m.p. 143–149 °C. IR (KBr), ν_{max}, cm^{-1}: 1669 (C=O), 1611 (C=C), 1594 (C=C), 1495 (C=C), 1209 (P=O), 1023 (P–O). ^{31}P NMR ($CDCl_3$, 121 MHz): δ = 22.63. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 0.98$ and 1.14 (both *t*, 6H, $^{3}J_{HH} = 7.1$ Hz, $P(OCH_2C\underline{H}_3)_2$), 3.60–3.99 (m, 31H, $POCH_2 + OCH_3 + CH_2$ (cyclic)), 4.00–4.04 (m, 2H, $POCH_2$), 4.09–4.14 (m, 2H, CH_2 (cyclic)), 4.62 (d, 1H, CHP, $^{2}J_{PH} = 23$ Hz), 6.64–6.67 (m, 3H), 6.97 (d, 2H, $^{3}J_{HH} = 8.7$ Hz), 7.51 (dd, 1H, $^{3}J_{HH} = 8.4$ Hz, $^{4}J_{HP} = 5.4$ Hz), 7.90 (s, 2H, $C^{14}H$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.2$ and 16.3 (both d, $^{3}J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 53.2 (d, $^{3}J_{PC} = 9$ Hz, C^2, C^6), 55.9 (C^{11}–O*Me*), 56.0 (C^{18}–O*Me*), 57.8 (d, $^{1}J_{PC} = 165$ Hz, C^7), 60.7 (C^{12}–O*Me*), 60.9 (C^{17}–O*Me*), 61.2 (C^{13}–O*Me*), 61.5 (C^{16}–O*Me*), 62.1 and 63.2 (both d, $^{2}J_{PC} = 7$ Hz, $POCH_2$), 106.7 (C^{10}), 106.8 (C^{19}), 117.6 (C^8), 122.4 (C^{15}), 125.2 (C^{20}), 125.6 (d, $^{3}J_{PC} = 4$ Hz, C^9), 130.7 (C^{14}), 132.9 (C^3, C^5), 142.0 (C^{12}), 142.3 (C^{17}), 152.9 (C^{11}), 153.0 (C^{13}), 153.7 (C^{18}), 154.7 (C^{16}), 187.3 (C^4). Anal. Calcd. for $C_{39}H_{50}NO_{13}P$ (%): C, 60.69; H, 6.53; N, 1.81. Found (%): C, 60.67; H, 6.59; N, 1.79.

*Diethyl (3,5-bis(3-hydroxybenzylidene)-4-oxopiperidin-1-yl)(4-fluorophenyl)methylphosphonate (**2n**)*

Piperidone **1b** (0.51 g; 1.5 mmol) was mixed with 3-hydroxybenzaldehyde (0.37 g; 3.0 mmol) in anhydrous CH_3CN (2 mL). Boron trifluoride etherate (1.28 g, 9.0 mmol) was added to the stirred mixture and stirring was continued for 48 h at RT. Then, Et_2O (25 mL) was added to the reaction mixture resulting in precipitation of sticky material. The precipitated material was washed with Et_2O (10 mL) followed by stirring with H_2O (20 mL) for 6 h until suspension of powder product was formed. The product was filtered off, resuspended in H_2O (40 mL) and 9 mL of aqueous NH_3 were added. Stirring was continued for 1 h followed by addition of glacial CH_3COOH (ca. 5 mL) to adjust pH to ca. 8. Solid substance was filtered off, dried in air and subjected to SiO_2 column chromatography. Elution was started with a 1:3 acetone/petroleum ether mixture and continued with a 3:2 acetone/petroleum ether mixture. Evaporation of eluates gave yellow crystalline product (0.24 g, 29 %), decomp. >185 °C. IR (KBr), ν_{max}, cm^{-1}: 3275 (OH), 1665 (C=O), 1606 (C=C), 1592 (C=C), 1579 (C=C), 1211 (P=O), 1020 (P–O). ^{31}P NMR (DMSO-d_6, 162MHz): $\delta = 21.86$. ^{19}F NMR (DMSO-d_6, 376 MHz): $\delta = -113.90$. ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 0.95$ and 1.11 (both *t*, 6H, $^3J_{HH} = 7.0$ Hz, P($OCH_2C\underline{H}_3)_2$), 3.51 (m, 1H, $POCH_2$), 3.63–3.72 (m, 5H, $POCH_2 + CH_2$ (cyclic)), 4.12–4.17 (m, 2H, CH_2 (cyclic)), 4.54 (d, 1H, CHP, $^2J_{PH} = 21$ Hz), 6.84–6.89 (m, 6H), 7.21–7.32 (m, 4H), 7.50 (s, 2H, C^7H), 7.59 (*t*, 2H, $^3J_{HH} = 6$ Hz), 9.81 (br s, 2H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.4$ and 16.6 (both d, $^3J_{PC} = 6$ Hz, $POCH_2\underline{C}H_3$), 52.9 (d, $^3J_{PC} = 8$ Hz, C^2, C^6), 62.3 and 62.7 (both d, $^2J_{PC} = 7$ Hz, $POCH_2$), 63.8 (d, $^1J_{PC} = 157$ Hz, C^7), 115.5 (d, $^2J_{FC} = 21$ Hz, C^{10}, C^{12}), 116.8 (C^{16}), 117.4 (C^{18}), 121.7 (C^{20}), 129.2 (C^8), 130.1 (C^{19}), 132.6 (*t*, $^3J_{FC} = {}^3J_{PC} = 8$ Hz, C^9, C^{13}), 134.1, 135.4 (C^3, C^5), 136.3 (C^{14}), 157.8 (C^{17}–OH), 162.4 (d, $^1J_{CF} = 244$ Hz, C^{11}), 187.1 (C^4). Anal. Calcd. for $C_{30}H_{31}FNO_6P$ (%): C, 65.33; H, 5.67; N, 2.54. Found (%): C, 65.33; H, 5.69; N, 2.45.

*3,5-Bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl)(4-fluorophenyl)methylphosphonic acid (**3**)*

Phosphonate **2e** (0.83 g; 1.5 mmol) was dissolved in methylene chloride (7 mL) in a flask. Bromotrimethylsilane (0.92 g; 6.0 mmol; 4 eq.) was added to the solution and the flask was stopped and kept in dark at room temperature for 7 days. Volatiles were removed under reduced pressure, mixture of MeOH/H_2O (5 mL : 5 mL) was added to the residue and the product was triturated to give yellow powder which was filtered off and dried in air to afford 0.75 g (100 %) of acid **3** as yellow powder, decomp. >220 °C. ^{31}P NMR (DMSO-d_6, 162 MHz): $\delta = 16.68$. ^{19}F NMR (DMSO-d_6, 376 MHz): $\delta = -110.97$ (F–C^{18}), -115.00 (F–C^{11}). ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 3.87$–3.91 (m, 2H, CH_2 (cyclic)), 4.00 (br s, 3.33H from $P(OH)_2$ and solvated H_2O overlapped with water in DMSO), 4.12 (d, 1H, $^2J_{PH} = 20$ Hz, CHP), 4.14–4.17 (m, 2H, CH_2 (cyclic)), 7.14 (*t*, 2H, $^3J_{HH} = {}^3J_{HF} = 8.0$ Hz, $C^{10}H$ and $C^{12}H$), 7.29 (*t*, 4H, $^3J_{HH} = {}^3J_{HF} = 8.0$ Hz, $C^{17}H$ and $C^{19}H$), 7.49–7.52 (m, 6H, $C^{16}H$ and $C^{20}H$, C^9H and $C^{13}H$), 7.54 (s, 2H, $C^{14}H$). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 52.7$ (C^2, C^6), 65.7 (d, $^1J_{PC} = 149$ Hz, C^7), 115.1 (d, $^2J_{FC} = 21$ Hz, C^{10}, C^{12}), 116.2 (d, $^2J_{FC} = 22$ Hz, C^{17}, C^{19}), 131.1 (d, $^4J_{FC} = 2$ Hz, C^8), 131.5 (d, $^4J_{CF} = 2$ Hz, C^{15}), 132.4 (br s, C^9, C^{13}), 133.2 (br s, C^{16}, C^{20}), 133.7 (C^3, C^5), 134.4 (C^{14}), 162.0 (d, $^1J_{CF} = 242$ Hz, C^{11}), 162.8 (d, $^1J_{CF} = 247$ Hz, C^{18}), 187.0 (C^4). Anal. Calcd. for $C_{26}H_{21}F_3NO_4P \times 2/3H_2O$ (%): C, 61.06; H, 4.40; N, 2.74. Found (%): C, 61.00; H, 4.32; N, 2.75.

*Monoammonium 3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl)(4-fluorophenyl)methylphosphonate (**4**)*

Phosphonic acid **3** (0.19 g; 0.38 mmol) was suspended in anhydrous EtOH (7 mL). Aqueous ammonia (ca. 25 % NH_3, 2 mL) was added to the magnetically stirred suspension, and the reaction was allowed to proceed for 1 h. The reaction mixture was evaporated to dryness under reduced pressure at RT. EtOH (4 mL) was added to the residue and evaporation was repeated to give ammonium salt as yellow powder (0.19 g, ca. 100 %), decomp. >220 °C. ^{31}P NMR (DMSO-d_6, 162 MHz): $\delta = 13.33$. ^{19}F NMR (DMSO-d_6, 376 MHz): $\delta = -110.31$ (F-C^{18}), -116.33 (F-C^{11}). ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.06$ (*t*, 0.75H, $^3J_{HH} = 7$ Hz, CH_3 (EtOH)), 3.44 (q, 0.5H, $^3J_{HH} = 6$ Hz, CH_2 (EtOH)), 3.77 (d, 1H, $^2J_{PH} = 20$ Hz, CHP), 4.00 (br s, 7.25H from NH_4, POH and solvated Et$O\underline{H}$ and H_2O overlapped with water in DMSO), 4.04–4.15 (m, 4H, CH_2 (cyclic)), 6.90 (*t*, 2H, $^3J_{HH} = {}^3J_{HF} = 8.7$ Hz, $C^{10}H$ and $C^{12}H$), 7.20 (*t*, 4H, $^3J_{HH} = {}^3J_{HF} = 8.7$ Hz, $C^{17}H$ and $C^{19}H$), 7.44–7.49 (m, 8H). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 19.0$ (EtOH), 53.3 (C^2, C^6), 56.4 (EtOH), 67.8 (d, $^1J_{PC} = 138$ Hz, C^7), 114.3 (d, $^2J_{FC} = 20$ Hz, C^{10}, C^{12}), 116.1 (d, $^2J_{FC} = 22$ Hz, C^{17}, C^{19}), 131.5 (d, $^2J_{FC} = 2$ Hz, C^8), 131.7 (d, $^4J_{CF} = 2$ Hz, C^{15}), 132.9 (C^9, C^{13}), 133.2 ($^3J_{CF} = 8$ Hz, C^{16}, C^{20}), 135.2 (C^3, C^5), 136.0 (C^{14}), 161.2 (d, $^1J_{CF} = 241$ Hz, C^{18}), 162.6 (d, $^1J_{CF} = 247$ Hz, C^{11}), 187.9 (C^4). Anal. Calcd. for $C_{26}H_{24}F_3N_2O_4P \times 1/4EtOH \times H_2O$ (%): C, 58.30; H, 5.08; N, 5.13. Found (%): C, 58.29; H, 5.11; N, 4.98.

X-ray structure determination of 2e

The single crystals of **2e** suitable for X-ray experiments were obtained by slow diffusion of hexane into a dichloromethane solution of the compound. Data were collected on

Table 2 Crystallographic data for **2e**

Empirical formula	$C_{30}H_{29}F_3NO_4P$
Fw	555.51
Crystal system	Triclinic
Space group	$P-1$
a, Å	7.5347(3)
b, Å	12.1065(5)
c, Å	15.3144(7)
α, deg.	95.317(1)
β, deg.	97.041(1)
γ, deg.	103.585(1)
V, Å^3	1336.91(10)
Z	2
d_{calc}, g·cm^{-3}	1.380
μ, mm^{-1}	0.161
$F(000)$	580
Crystal size, mm	$0.25 \times 0.15 \times 0.15$
$2\theta_{max}$, deg.	60.0
Reflections collected	17,030
Independent reflections	7781
Reflections observed with $I > 2\sigma(I)$	6589
Data/restraints/parameters	7781/6/371
GOF on F^2	1.078
R_1 [$I > 2\sigma(I)$]	0.0395
wR_2 [all data]	0.1106
Largest diff. peak and hole	0.523/−0.389
T_{min}/T_{max}	0.961/0.976

a Bruker APEX II CCD diffractometer ($T = 120.0(2)$ K, λ (MoK_α)-radiation, graphite monochromator, ω and φ scan mode) and corrected for absorption using the *SADABS* program. For details, see Table 2. The crystal structure was determined by direct methods and refined by the full-matrix least squares technique on F^2 with anisotropic displacement parameters for non-hydrogen atoms. The both ethyl groups in **2e** are disordered over two sites each, with the same occupancies of 0.6:0.4. The hydrogen atoms were placed in calculated positions and refined within the riding model with fixed isotropic displacement parameters ($U_{iso}(H) = 1.5U_{eq}(C)$ for the CH_3-groups and $U_{iso}(H) = 1.2U_{eq}(C)$ for the other groups). All calculations were carried out using the *SHELXTL* program. Crystallographic data for **2e** have been deposited with the Cambridge Crystallographic Data Center. CCDC 1457965 contains supplementary crystallographic data for this paper. These data can be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk).

Biological evaluations

Cell lines used for estimation of toxicity of the compounds were HCT116 (colon cancer), MCF7 (breast cancer), RD (rhabdomyosarcoma), PC3 (prostate cancer) as well as HEK293 (human embryonic kidney cells). 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 colorimetric assay. The test is based on the ability of mitochondrial dehydrogenase in viable cells to convert MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (ICN Biomedicals, Germany) into a soluble blue formazan dye. The cell lines were seeded into 96-well plates at the 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 DMSO (Sigma Aldrich) and the working solutions were added to FBS free culture medium. The compounds were added to wells with increasing concentrations. After 72 h of 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 DMSO to determine the amount of formazan product. The optical density was determined by the multi-well plate reader (Uniplan, Picon, Russia) at 590 nm. The results were expressed as percent decrease in 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}, and 10^{-7} M. Commercially available Doxorubicin and Daunorubicin were used as positive controls in the assay.

Acknowledgments This work was supported by the Russian Foundation for Basic Research (project no. 14-03-00687).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB (2007) Bioavailability of curcumin: problems and promises. Mol Pharmaceutics 4:807–818

Anchoori RK, Karanam B, Peng S, Wang JW, Jiang R, Tanno T, Orlowski RZ, Matsui W, Zhao M, Rudek MA, Hung C, Chen X, Walters KJ, Roden RBS (2013) A bis-benzylidine piperidone targeting proteasome ubiquitin receptor RPN13/ADRM1 as a therapy for cancer. Cancer Cell 24:791–805

Arnold A, Markert M, Mahrwald R (2006) Amine-catalyzed aldol condensation in the presence of lithium perchlorate. Synthesis 38:1099–1102

Bazzaro M, Anchoori RK, Mudiam MKR, Issaenko O, Kumar S, Karanam B, Lin Z, Vogel RI, Gavioli R, Destro F, Ferretti V, Roden RBS, Khan SR (2011) α,β-Unsaturated carbonyl system of chalcone-based derivatives is responsible for broad inhibition of proteasomal activity and preferential killing of human papilloma virus (HPV) positive cervical cancer cells. J Med Chem 54:449–456

Das U, Sharma RK, Dimmock JR (2009a) 1,5-Diaryl-3-oxo-1,4-pentadienes: a case for antineoplastics with multiple targets. Curr Med Chem 16:2001–2020

Das S, Das U, Selvakumar P, Sharma RK, Balzarini J, De Clercq E, Molnár J, Serly J, Baráth Z, Schatte G, Bandy B, Gorecki DKJ, Dimmock JR (2009b) 3,5-Bis(benzylidene)-4-oxo-1-phosphonopiperidines and related diethyl esters: potent cytotoxins with multidrug-resistance reverting properties. Chem Med Chem 4:1831–1840

Das S, Das U, Sakagami H, Hashimoto K, Kawase M, Gorecki DKJ, Dimmock JR (2010) Sequential cytotoxicity: a theory examined using a series of 3,5-bis(benzylidene)-1-diethylphosphono-4-oxopiperidines and related phosphonic acids. Bioog Med Chem Lett 20:6464–6468

Das S, Das U, Varela-Ramírez A, Lema C, Aguilera RJ, Balzarini J, De Clercq E, Dimmock SG, Gorecki DKJ, Dimmock JR (2011) Bis[3,5-bis(benzylidene)-4-oxo-1-piperidinyl]amides: a novel class of potent cytotoxins. ChemMedChem 6:1892–1899

Das S, Das U, Michel D, Gorecki DKJ, Dimmock JR (2013) Novel 3,5-bis(arylidene)-4-piperidone dimers: potent cytotoxins against colon cancer cells. Eur J Med Chem 64:321–328

Dimmock JR, Padmanilayam MP, Puthucode RN, Nazarali AJ, Motaganahalli NL, Zello GA, Quail JW, Oloo EO, Kraatz HB, Prisciak JS, Allen TM, Santos CL, Balzarini J, De Clercq E, Manavathu EK (2001) A conformational and structure-cctivity relationship study of cytotoxic 3,5-bis(arylidene)-4-piperidones and related N-acryloyl analogues. J Med Chem 44:586–593

Ezra A, Hoffman A, Breuer E, Alferiev IS, Mönkkönen J, El Hanany-Rozen N, Weiss G, Stepensky D, Gati I, Cohen H, Törmälehto S, Amidon GL, Golomb G (2000) A peptide prodrug approach for improving bisphosphonate oral absorption. J Med Chem 43:3641–3652

Gregory M, Dandavati A, Lee M, Tzou S, Savagian M, Brien KA, Satam V, Patil P, Lee M (2013) Synthesis, cytotoxicity, and structure-activity insight of *NH*- and N-methyl-3,5-bis-(arylidenyl)-4-piperidones. Med Chem Res 22:5588–5597

Jha A, Mukherjee C, Prasad AK, Parmar VS, De Clercq E, Balzarini J, Stables JP, Manavathu EK, Shrivastav A, Sharma RK, Nienaber KH, Zello GA, Dimmock JR (2007) *E,E,E*-1-(4-Arylamino-4-oxo-2-butenoyl)-3,5-bis(arylidene)-4-piperidones: a topographical study of some novel potent cytotoxins. Bioorg Med Chem 15:5854–5865

Kálai T, Kuppusamy ML, Balog M, Selvendiran K, Rivera BK, Kuppusamy P, Hideg K (2011) Synthesis of *N*-substituted 3,5-bis (arylidene)-4-piperidones with high antitumor and antioxidant activity. J Med Chem 54:5414–5421

Kudo C, Yamakoshi H, Sato A, Ohori H, Ishioka C, Iwabuchi Y, Shibata H (2011) Novel curcumin analogs, GO-Y030 and GO-Y078, are multitargeted agents with enhanced abilities for multiple myeloma. Anticancer Res 31:3719–3726

Ledoux D, Hamma-Kourbali Y, Di Benedetto M, Foucault-Bertaud A, Oudar O, Sainte-Catherine O, Lecouvey M, Kraemer (2006) A new dimethyl ester bisphosphonate inhibits angiogenesis and growth of human epidermoid carcinoma xenograft in nude mice. Anti-Cancer Drugs 17:479–485

Leonova E, Makarov M, Klemenkova Z, Odinets I (2010) Lewis acids as mild and effective catalysts for the synthesis of 3,5-bis[(hetero) arylidene]piperidin-4-ones. Helv Chim Acta 93:1990–1999

Makarov MV, Rybalkina EYu, Röschenthaler GV, Short KW, Timofeeva TV, Odinets IL (2009) Design, cytotoxic and fluorescent properties of novel *N*-phosphorylalkyl substituted *E,E*-3,5-bis(arylidene)piperid-4-ones. Eur J Med Chem 44:2135–2144

Makarov MV, Leonova ES, Rybalkina EYu, Khrustalev VN, Shepel NE, Röschenthaler GV, Timofeeva TV, Odinets IL (2012) Methylenebisphosphonates with dienone pharmacophore: synthesis, structure, antitumor and fluorescent properties. Arch Pharm Chem Life Sci 345:349–359

Makarov MV, Skvortsov EA, Brel VK (2015) Synthesis of diethyl (aryl)(4-oxopiperidin-1-yl)methylphosphonates. Mendeleev Comm 25:232–233

Mosley CA, Liotta DC, Snyder JP (2007) Highly active anticancer curcumin analogues. Adv Exp Med Biol 595:77–103

Patel MR, Dimmock JR, Talele TT (2007) CoMFA and CoMSIA studies on 1,3-bis(benzylidene)-3,4-dihydro-1*H*-naphthalen-2-one, 2,6-bis(benzylidene)cyclohexanone, and 3,5-bis(benzylidene)-4-piperidone series of cytotoxic compounds. J Chem Inf Model 47:2110–2123

Porras AG, Gertz BJ (2004) The role of clinical pharmacology and of pharmacokinetics in the development of alendronate – a bone resorption inhibitor. In: Krishna R (ed) Applications of pharmacokinetic principles in drug development, 1st edn. Springer Science+Business Media, New York, p 427–475

Santiago-Vazquez Y, Das S, Das U, Robles-Escajeda E, Ortega NM, Lema C, Varela-Ramírez A, Aguilera RJ, Balzarini J, De Clercq E, Dimmock SG, Gorecki DKJ, Dimmock JR (2014) Novel 3,5-bis(arylidene)-4-oxo-1-piperidinyl dimers: structure-activity relationships and potent antileukemic and antilymphoma cytotoxicity. Eur J Med Chem 77:315–322

Selvendiran K, Kuppusamy ML, Bratasz A, Tong L, Rivera BK, Rink C, Sen CK, Kálai T, Hideg K, Kuppusamy P (2009) Inhibition of vascular smooth-muscle cell proliferation and arterial restenosis by HO-3867, a novel synthetic curcuminoid, through up-regulation of PTEN expression. J Pharmacol Exp Ther 329:959–966

Shetty D, Kim YJ, Shim H, Snyder JP (2015) Eliminating the heart from the curcumin molecule: monocarbonyl curcumin mimics (MACs). Molecules 20:249–292

Shehzad A, Wahid F, Lee YS (2010) Curcumin in cancer chemoprevention: molecular targets, pharmacokinetics, bioavailability, and clinical trials. Arch Pharm Chem Life Sci 343:489–499

Shibata H, Iwabuchi Y (2012) Challenges in establishing potent cancer chemotherapy using newly synthesized 1,5-diaryl-3-oxo-1,4-pentadiene analogs of curcumin. In: Sasaki J, Kichida M (eds) Curcumin: biosynthesis, medicinal uses and health benefits (Food science and technology). Nova Science Publishers, New York, pp 177–192

Singh RS, Michel D, Das U, Dimmock JR, Alcorn J (2014) Cytotoxic 1,5-diaryl-3-oxo-1,5-pentadienes: an assessment and comparison of membrane permeability using Caco-2 and MDCK monolayers. Bioorg Med Chem Lett 24:5199–5202

Sun J, Zhang S, Yu C, Hou G, Zhang X, Li K, Zhao F (2014) Design, synthesis and bioevaluation of novel *N*-substituted-3,5-bis(arylidene)-4-piperidone derivatives as cytotoxic and antitumor agents with fluorescent properties. Chem Biol Drug Des 83:392–400

Thakur A, Manohar S, Gerena CEV, Zayas B, Kumar V, Malhotra SV, Rawat DS (2014) Novel 3,5-bis(arylidiene)-4-piperidone based monocarbonyl analogs of curcumin: anticancer activity evaluation and mode of action study. Med Chem Commun 5:576–586

Vyas A, Dandawate P, Padhye S, Ahmad A, Sarkar F (2013) Perspectives on new synthetic curcumin analogs and their potential anticancer properties. Curr Pharm Des 19:2047–2069

Youssef KM, El-Sherbeny MA (2005) Synthesis and antitumor activity of some curcumin analogs. Arch Pharm Chem Life Sci 338:181–189