Modification of 3,5-bis(arylidene)-4-piperidone pharmacophore by phosphonate group using 1,2,3-triazole cycle as a linker for the synthesis of new cytostatics

Mikhail V. Makarov, Ekaterina Yu. Rybalkina, Victor N. Khrustalev & Gerd-Volker Röschenthaler

Medicinal Chemistry Research

ISSN 1054-2523
Volume 24
Number 4

Med Chem Res (2015) 24:1753-1762
DOI 10.1007/s00044-014-1262-z

Springer

Med Chem Res (2015) 24:1753–1762
DOI 10.1007/s00044-014-1262-z

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Modification of 3,5-bis(arylidene)-4-piperidone pharmacophore by phosphonate group using 1,2,3-triazole cycle as a linker for the synthesis of new cytostatics

Mikhail V. Makarov · Ekaterina Yu. Rybalkina · Victor N. Khrustalev · Gerd-Volker Röschenthaler

Received: 14 June 2014 / Accepted: 13 September 2014 / Published online: 20 September 2014

Abstract Novel cytotoxic 3,5-bis(arylidene)-4-piperidones conjugated with phosphonate groups via 1,2,3-triazole ring have been synthesized and their antitumor properties have been evaluated. Synthetic route to these conjugates is based on 1,3-cycloaddition of diethyl (ω-azidoalkyl)phosphonates to 1-prop-2-ynyl-piperidin-4-one in the presence of Cu(I) catalyst followed by crotonic condensation of resulting 1,2,3-triazole with aromatic aldehydes. The synthesized phosphonate derivatives of 3,5-bis(arylidene)-4-piperidone series displayed high in vitro inhibitory properties toward HCT116 and MCF7 as well as CaoV3, A549, and PC3 human cancer cell lines with IC_{50} values in the range of 1.5–8.0 μM.

Keywords 3,5-Bis(arylidene)-4-piperidones · Antitumor activity · Aminophosphonates · 1,2,3-Triazoles · 1,3-Cycloaddition

Introduction

3,5-Bis(arylidene)-4-piperidones **I** (Scheme 1) contain conjugated 1,5-diaryl-3-oxo-1,4-pentadienyl (dienone) pharmacophore responsible for their anticancer properties (Das *et al*., 2009). Even though the mechanism of antitumor activity of these compounds is not fully understood, it is believed that the ability of vinyl bonds of the dienone pharmacophore to alkylate intracellular thiols plays an essential role in inhibiting cancer cells by such compounds. For example, it was shown that vinyl bonds of dienone moiety were able to covalently bind to cysteine 88 of RPN13 ubiquitin receptor, which resulted in triggering rapid accumulation of polyubiquitinated proteins and death of cancer cells (Anchoori *et al*., 2013). Moreover, because vinyl bonds of the pharmacophore do not affect hydroxyl- and nitrogen-containing cellular nucleophiles, such as nucleic acids, 3,5-bis(arylidene)-4-piperidones may be devoid of genotoxicity characteristic of conventional alkylating agents currently applied in clinical practice (Das *et al*., 2009).

On the other hand, based on the structural resemblance of compounds with dienone pharmacophore to curcumin also revealing antitumor properties, one may assume that 3,5-bis(arylidene)-4-piperidones may affect the same biological targets as curcumin (Mosley *et al*., 2007). One of the disadvantages of curcumin is that it is difficult to modify its structure to increase the activity, whereas 3,5-bis(arylidene)-4-piperidones provide possibilities for such type of modification.

The main way for structural modification of 3,5-bis(arylidene)-4-piperidones to change their biological properties consists in introducing various groups R^1 (Scheme 1) to the piperidone nitrogen atom. These groups may have their own biological activity, change pharmacological properties of the

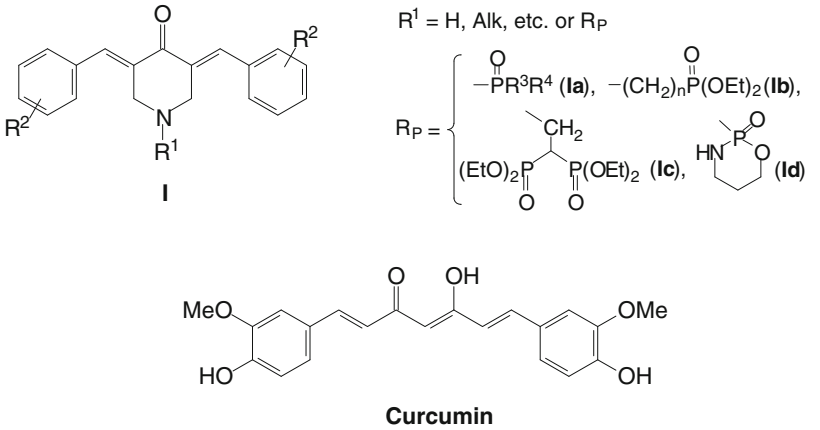
Electronic supplementary material The online version of this article (doi:10.1007/s00044-014-1262-z) contains supplementary material, which is available to authorized users.

M. V. Makarov (✉) · V. N. Khrustalev
A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 119991 Moscow, Russian Federation
e-mail: mmak78@yandex.ru

E. Yu. Rybalkina
Institute of Carcinogenesis, N. N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences, 115478 Moscow, Russian Federation

G.-V. Röschenthaler
Jacobs University Bremen GmbH, 28759 Bremen, Germany

Scheme 1 Structures of known 3,5-bis(arylidene)-4-piperidones, including those modified by phosphorus-containing groups, and curcumin



molecule influencing the delivery of dienone pharmacophore to a biological target, or interact with an additional binding site of a potential receptor. For instance, sulfonamide (Thakur *et al*., 2014), nitroxide (Kálai *et al*., 2011), and amino acid residues (Bazzaro *et al*., 2011) were recently described as such modifying groups. It should be also noted that phosphorus-containing moieties, in particular residues of phosphoric (phosphonic) acids of various structures, belong to advantageous groups allowing for regulating antitumor properties of biologically active compounds and drugs. For example, well-known phosphorus-containing cytostatic cyclophosphan contains oxazaphosphorinane cycle attached to the nitrogen mustard residue. Moreover, bisphosphonate moiety was used in a number of publications to modify some antitumor drugs such as gemcitabine (El-Mabhouh and Mercer, 2008), methotrexate (Sturtz *et al*., 1993), doxorubicin (Fabulet and Sturtz, 1995), and nucleoside antimetabolites (Reinholz *et al*., 2010).

In our previous works, we elaborated approaches to the synthesis of phosphorus acid amides **Ia** (Odinets *et al*., 2005; Makarov *et al*., 2010; Leonova *et al*., 2010a), aminophosphonates **Ib** (Makarov *et al*., 2009), bisphosphonates **Ic** (Makarov *et al*., 2012), as well as oxazaphosphorinane derivatives **Id** (Shipov *et al*., 2013) bearing 3,5-bis(arylidene)-4-piperidone framework and showed that attaching phosphorus-containing groups to the bis(arylidene)piperidone pharmacophore did indeed make it possible to increase their antitumor activity. An increase in cytotoxic activity as a result of *N*-phosphorylation of *NH*-3,5-bis(arylidene)-4-piperidones was also noted in work of Das et al., 2010. Furthermore, we have recently demonstrated that some phosphorus-containing 3,5-bis(arylidene)-4-piperidones are not substrates for the Pgp170 glycoprotein (Leonova *et al*., 2010a) responsible for removal of hydrophobic compounds, including cytostatics, from tumor cells and, therefore, responsible for multidrug resistance which is one of the causes for decreasing efficiency of antitumor chemotherapy. For this reason, variation of phosphorus-containing groups and a linker connecting phosphorus atom of such groups with the piperidone residue is an important approach for the development of 3,5-bis(arylidene)-4-piperidones with increased cytotoxic activity.

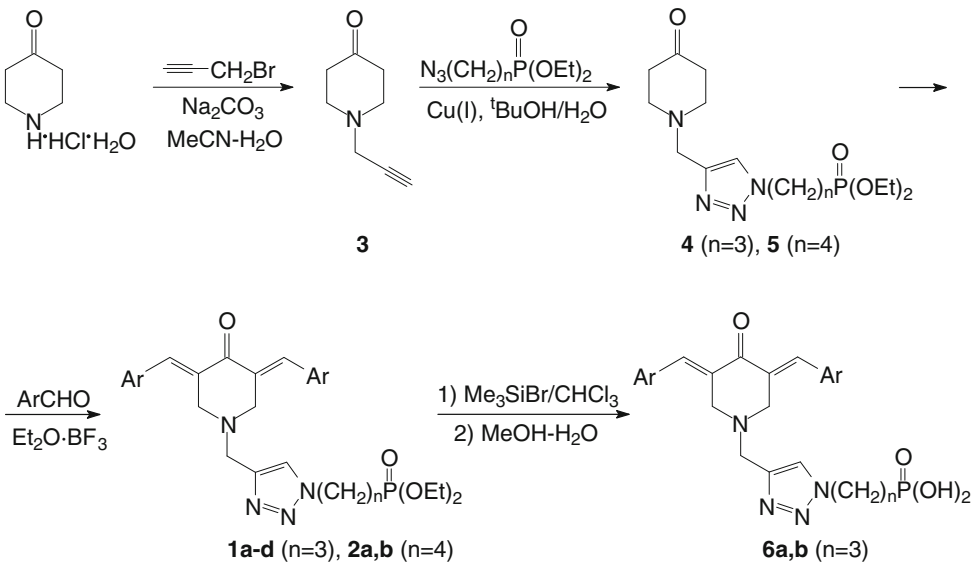
Previously, to conjugate phosphorus-containing moieties with organic compounds, including biologically active ones, an approach based on copper(I)-catalyzed 1,3-dipolar cycloaddition of organic azides to acetylenes (Huisgen reaction) resulting in formation of 1,2,3-triazole ring as a linker between a phosphorus and organic component was successfully used in our laboratories (Skarpos *et al*., 2007; Artyushin *et al*., 2009). The catalyzed version of Huisgen reaction is well known to be distinguished by a number of advantages as compared to the standard procedure for conducting this transformation. Indeed, in the presence of Cu(I) the reaction may be carried out under milder conditions and proceeds regioselectively resulting in the formation of exclusively 4-regioisomers of the dipolar cycloaddition products making it possible to purposefully link various moieties in a target molecule.

Taking into account the above advantageous features of Cu(I) catalyzed 1,3-dipolar Huisgen cycloaddition, the possibility of conjugation of 3,5-bis(arylidene)-4-piperidone pharmacophore with phosphonate group through 1,2,3-triazole ring formed as a result of reaction of acetylenes bearing a piperidone residue with phosphonate-containing azido groups was studied, and synthetic approaches to corresponding phosphonates **1** and **2** of 3,5-bis(arylidene)-4-piperidone series (Scheme 2) were elaborated in the present work.

Results and discussion

Synthesis and structure

To prepare target compounds **1** and **2** (differing in the length of alkylene chain connecting phosphonate group



Scheme 2 Synthesis of 3,5-bis(arylidene)-4-piperidones **1a–d,** and **2a**, **b** modified by phosphonate residues using 1,2,3-triazole cycle as a linker

Ar = 4-$O_2NC_6H_4$ (**a**), 4-NCC_6H_4 (**b**), 4-FC_6H_4 (**c**), 4-$MeOC_6H_4$ (**d**)

with nitrogen atom of 1,2,3-triazole ring), we used an approach including the synthesis of terminal acetylene **3** followed by 1,3-cycloaddition of phosphorylated azide thereto resulting in product **4** or **5** which was finally used in aldol-crotonic condensation with aromatic aldehydes (Scheme 2).

According to this approach, the first step of the synthetic sequence included alkylation of commercially available 4-piperidone hydrochloride monohydrate with propargyl bromide in a water/acetonitrile medium in the presence of sodium carbonate as a base. Desired acetylene **3** was obtained in a satisfactory yield (59 %) after column chromatography. 1,3-Cycloaddition reaction of diethyl(3-azidopropyl)phosphonate with this acetylene in the presence of univalent copper generated in situ by reduction of Cu(II) with sodium ascorbate in the 4:1 ^{t}BuOH/H_2O system proceeded practically quantitatively to give corresponding triazole **4** with purity of about 98 % (according to ^{1}H and ^{31}P NMR). At the same time, an analogous reaction of diethyl(4-azidobutyl)phosphonate gave somewhat worse result (according to ^{1}H NMR, the reaction product contained starting acetylene in the amount of 12 %) that may be probably accounted for by lower reactivity of azidobutylphosphonate. Purification of crude reaction product using column chromatography afforded desired compound **5** in the yield of 65 %.

In the last step, aldol-crotonic condensation of ketones **4** and **5** with aromatic aldehydes was carried out in the presence of excess of boron trifluoride etherate in accordance with the procedure elaborated in our previous work (Leonova *et al.*, 2010b). According to the ^{1}H NMR data, crude reaction products contained *E,E*-isomers of corresponding 3,5-bis(arylidene)-4-piperidones as main components, admixtures of starting substances, as well as small amounts of compounds which we believe to be *E,Z*- and/or *Z,Z*-isomers of 3,5-bis(arylidene)-4-piperidones. The presence of these admixtures necessitated using recrystallization (precipitation) besides column chromatography to purify the target phosphonates **1a–d**, **2a**, **b** which were eventually obtained in the yield of 45–61 %. Finally, the ester products can be converted into corresponding free phosphonic acids by reacting with trimethylbromosilane followed by methanolysis, as illustrated by phosphonates **1a**, **b** as representative examples (Scheme 2).

The structures of the phosphorylated products **1a–d**, **2a**, **b** were elucidated by ^{1}H, ^{31}P, and ^{13}C NMR and IR spectral data. The ^{31}P NMR spectra of these phosphonates displayed the singlet signals observed at 29.79–30.02 ppm for compounds **1a–d** and 30.73–30.81 ppm for compounds **2a**, **b** that are typical values for diethyl phosphonates. In the ^{1}H NMR spectra of all compounds, the singlet resonances assigned to the sole hydrogen atom of 1,2,3-triazole moiety were observed at 7.38–7.48 ppm while in ^{13}C NMR spectra the carbon atom connected with this hydrogen resonated at 122.41–122.92 ppm. The quaternary carbon atom of 1,2,3-triazole ring had a ^{13}C chemical shift at 143.14–144.16 ppm depending on the particular compound. *E,E*-configuration of vinyl bonds in dienone moiety of phosphonates **1a–d**, **2a**, **b** was established by comparing their NMR spectra with those of known corresponding *NH*-3,5-bis(arylidene)-4-piperidones. The IR spectra of **1a–d**, **2a**, **b** show characteristic absorption bands at 1240–1255 (P=O), 1024–1032 (P–O–C), and 1668–1677 (C=O) cm^{-1}.

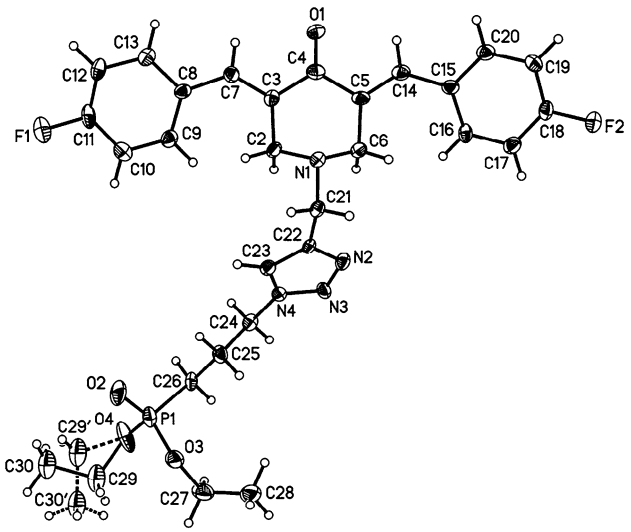


Fig. 1 Molecular structure of **1c** drawn at 40 % probability of anisotropic displacement ellipsoids. The alternative position of the disordered ethoxy group is depicted by dashed line

The structure of fluorine derivative **1c**, wherein phosphorus atom and triazole linker are separated by three methylene units, was also elucidated by X-ray diffraction analysis of a single crystal (Fig. 1).

The main backbone of molecule **1c** contains three planar fragments; the first includes the basal plane of the piperidone cycle (P_A), while the planar fragments P_B and P_C include a benzene ring and adjacent atoms. The dihedral angles P_A/P_B and P_A/P_C between these fragments are equal to 21.1° and 32.4°, 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 0.692 Å. The N1 nitrogen atom of the piperidone ring has trigonal–pyramidal configuration (sum of bond angles at the N1 atom is 333.9°).

The triazole-phosphonate ligand occupies the more sterically favorable equatorial position. The interplane angle between the basal plane of the piperidone ring and the triazole ring plane is 45.2°. 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 66.8(5)°.

Cytotoxic properties

The cytotoxic activity of the compounds **1a–d**, **2a**, **b** and **6a**, **b** was tested in vitro against human cancer cell lines, namely HCT116 (colon cancer) and MCF7 (breast cancer), and normal human embryonic fibroblast (HEF) cells. Compounds **1a** and **1c** were also evaluated for their cytotoxicity toward CaoV3 (ovarian cancer), A549 (lung cancer), and PC3 (prostate cancer) cell lines. The results are summarized in Tables 1 and 2 showing the corresponding IC_{50} values (IC_{50} is the concentration of compound required to inhibit the growth of the cells by 50 %). Anticancer antibiotic Doxorubicin was used as a positive control.

Table 1 Cytotoxicity of phosphonates **1a–d**, **2a**, **b** and free phosphonic acids **6a**, **b** toward human carcinoma cell lines HCT116 and MCF7 and normal fibroblasts HEF

Compound	IC_{50}, μM		
	HCT116	MCF7	HEF
1a	8.0 ± 0.45	1.75 ± 0.7	2.7 ± 0.4
1b	3.0 ± 0.4	2.1 ± 0.4	1.45 ± 0.25
1c	2.55 ± 0.75	1.7 ± 0.5	2.45 ± 0.6
1d	17.0 ± 2.2	15.0 ± 0.7	30.0 ± 3.5
2a	3.5 ± 1.1	2.75 ± 0.55	2.1 ± 0.4
2b	3.75 ± 0.25	2.5 ± 0.4	2.45 ± 0.55
6a	NA[a]	NA	NA
6b	NA	NA	NA
Doxorubicin	1.6 ± 0.3	0.3 ± 0.1	2.1 ± 0.4

[a] *NA* not active ($IC_{50} > 50$ μM)

Data in Table 1 indicate that MCF7 cells in general are more sensitive to compounds **1** and **2** as compared to HCT116 cells. With the single exclusion of compound **1d**, HCT116 cells proved to be more resistant to our antitumor agents than MCF7 and HEF cells. On the whole, representatives **1b**, **c** demonstrated the highest cytotoxicity. Compound **1d** containing electron-releasing methoxy groups in *para*-position of benzene rings had the lowest cytotoxicity, with normal HEF cells demonstrating higher resistance to it than cancer cells. Comparison between compounds **1** and **2** showed that there was no any dependence of cytotoxicity on the length of alkylene linker connecting phosphonate group with the rest of molecule. It should be noted that free phosphonic acids **6a**, **b** turned out to be non-toxic, probably due to their inability 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.

As it follows from Table 2, the IC_{50} values of compounds **1a**, **c** toward ovarian, lung, and prostate cancer cells are in the range of 1.5–7.5 μM, with CaoV3 cells being the most resistant. Here, a comparison of in vitro activity of aminophosphonates **1a**, **c** with that of structurally related compounds **Ib** ($n = 3$, Scheme 1), wherein phosphonate group is connected with piperidone cycle by

Table 2 Cytotoxicity of phosphonates **1a**, **c** toward human carcinoma cell lines CaoV3, A549 and PC3

Compound	IC_{50}, μM		
	CaoV3	A549	PC3
1a	7.5 ± 0.5	3.0 ± 1.2	1.5 ± 0.5
Ib-NO$_2$	5.62 ± 0.21	7.12 ± 0.41	0.71 ± 0.08
1c	5.5 ± 0.5	2.0 ± 0.5	5.0 ± 1.0
Ib-F	8.50 ± 0.03	9.07 ± 0.32	1.82 ± 0.44
Doxorubicin	2.7 ± 1.3	4.4 ± 1.1	1.4 ± 0.4

propylene chain without mediating methylene-1,2,3-triazole linker, was made. Compound **Ib-NO$_2$** ($n = 3$, $R^2 = 4$–NO_2) is an analog of **1a** and compound **Ib-F** ($n = 3$, $R^2 = 4$–F) is an analog of **1c**. Data for phosphonates **Ib-NO$_2$** and **Ib-F** were taken from our previous work (Makarov *et al.*, 2009). From the results presented, one may see that introduction of methylene-1,2,3-triazole linker results in noticeable increase (approximately three-fold) in cytotoxicity of **1a**, **c** in comparison with the non-triazole counterparts only in the case of A549 cell line. On the contrary, in the case of PC3 cells, compounds **Ib-NO$_2$** and **Ib-F** were more active than triazole-containing analogs. Finally, the difference in the activity between **1a** and **Ib-NO$_2$** as well as **1c** and **Ib-F** toward the CaoV3 cell line is not so evident.

In general, data of Tables 1 and 2 indicate that most of the compounds **1a–c**, **2a**, **b** have rather high cytotoxicity toward A549, PC3, HCT116, and MCF7 cancer cell lines demonstrating uniform IC_{50} values in the range of 1.5–8.0 μM. Doxorubicin was on the whole more active than arylidenepiperidones **1a–c**, **2a**, **b** but in the case of A549 and PC3 cell lines nitro and fluoro derivatives **1a**, **c** demonstrated comparable antitumor activity.

Conclusions

This study has demonstrated that 3,5-bis(arylidene)-4-piperidone pharmacophore may be modified by phosphonate groups using click approach based on the Huisgen reaction. The cytotoxicity screening revealed that most of the resulting phosphonates of 3,5-bis(arylidene)-4-piperidone series had rather high antitumor activity toward human cancer cell lines with IC_{50} values in a micromolar range (1.5–8.0 μM). Comparison with related phosphonates without 1,2,3-triazole ring between piperidone scaffold and phosphonate group showed that 1,2,3-triazole had not any significant negative impact on the antitumor activity of compounds and, on the contrary, a positive effect was observed for compounds **1a**, **c** in the case of A549 lung cancer cells.

Experimental

Chemistry

NMR spectra were recorded on a Bruker Avance 400 spectrometer (^{1}H, 400.13; ^{31}P, 161.97 and ^{13}C, 100.61 MHz) or Bruker Avance 300 spectrometer (^{1}H, 300.13; ^{19}F, 282.4; ^{31}P, 121.49 and ^{13}C, 75.47 MHz) using residual proton signal (^{1}H) and that of carbon atom (^{13}C) of a deuterated solvent as an internal standard relative TMS and CF_3COOH (^{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. 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; all solvents were reagent grade. The starting diethyl 3-azidopropylphosphonate and diethyl 4-azidobutylphosphonate were prepared according to published procedure (Artyushin *et al.*, 2008).

*1-Prop-2-ynyl-piperidin-4-one (**3**)*

Piperidone monohydrate hydrochloride (3.38 g, 0.022 mol) was mixed with a solution of Na_2CO_3 (4.57 g, 0.043 mol) in water (30 ml). The solution obtained was stirred at room temperature for 5 min and then a solution of propargyl bromide (2.38 g, 0.02 mol) in CH_3CN (30 ml) was added thereto. The reaction mixture was stirred at room temperature for 5 days, and then water and $CHCl_3$ were added. Organic phase was separated and aqueous phase was extracted with $CHCl_3$. Combined organic solutions were evaporated at reduced pressure leaving crude product as brownish oil (2.08 g). This was subjected to column chromatography purification (column 30 × 2 cm, elution was started with CH_2Cl_2 and continued with a mixture of CH_2Cl_2/MeOH = 100:1). Appropriate fractions were collected and evaporated at reduced pressure leaving light yellow oil (1.62 g, 59 %) of the desired compound. This oil transformed in a crystalline mass (m.p. 40.7–41.6 °C) after having been stored in refrigerator at −20 °C. IR (KBr), ν/cm^{-1}: 3238 (H–C≡), 2964, 2903, 2799, 1711 (C=O), 1679, 1477, 1444, 1397, 1369, 1352, 1328, 1316, 1290, 1277, 1244, 1221, 1208, 1128, 1117, 1092, 1019, 998, 991, 812, 762, 728, 682, 508, 437. ^{1}H NMR ($CDCl_3$,

300.13 MHz), δ: 2.25 (t, $^4J_{HH} = 2.4$ Hz, 1H, C≡CH), 2.44 (t, $^3J_{HH} = 6.2$ Hz, 4H), 2.82 (t, $^3J_{HH} = 6.2$ Hz, 4H), 3.39 (d, $^4J_{HH} = 2.4$ Hz, 2H, $C\underline{H}_2$–C≡CH). ^{13}C NMR ($CDCl_3$, 75.47 MHz), δ: 40.89 ($\underline{C}H_2CO\underline{C}H_2$), 46.17 ($\underline{C}H_2C\equiv CH$), 51.65 ($NC_{Pip}H_2$), 73.47 (C≡$\underline{C}$H), 78.07 ($\underline{C}\equiv CH$), 207.97 (C=O). Anal. calcd. for $C_8H_{11}NO$ (%): C, 70.04; H, 8.08; N, 10.21. Found (%): C, 70.25; H, 8.28; N 10.05.

*Diethyl 3-{4-[(4-oxopiperidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylphosphonate (**4**)*

1-Prop-2-ynyl-piperidin-4-one (1.66 g, 0.0121 mol) and diethyl 3-azidopropylphosphonate (2.68 g, 0.0121 mol) were mixed in a mixture of tert-BuOH/H_2O = 4:1 (35 ml). Then solid sodium ascorbate (0.72 g, 0.0036 mol) and solution of $CuSO_4{\cdot}5H_2O$ (0.15 g, 0.0006 mol) in H_2O (3 ml) were added in sequence to the reaction mixture. The reaction mixture was magnetically stirred at ambient temperature for 24 h, and then volatiles were removed using a rotary evaporator, the remainder was dissolved in $CHCl_3$, and solution obtained was washed with water. Organic phase was separated, dried over Na_2SO_4, filtered and evaporated at reduced pressure leaving light yellow oil (4.34 g, 100 %) of ~98 % purity according to ^{1}H NMR. This substance was used in further reactions without additional purification. Sample for elemental analysis was obtained using column chromatography (eluent: mixture of $CHCl_3$/MeOH, gradient from 100:3 to 10:1). ^{31}P NMR ($CDCl_3$, 161.97 MHz), δ: 29.93. ^{1}H NMR ($CDCl_3$, 400.13 MHz), δ: 1.28 (t, $^3J_{HH} = 7.1$ Hz, 6H, $POCH_2C\underline{H}_3$), 1.68 (dt, $^3J_{HH} = 7.7$ Hz, $^2J_{PH} = 18.6$ Hz, 2H, $NCH_2CH_2C\underline{H}_2P$), 2.14–2.25 (m, 2H, $NCH_2C\underline{H}_2CH_2P$), 2.40 (t, $^3J_{HH} = 6.0$ Hz, 4H, $NC_{Pip}H_2$), 2.77 (t, $^3J_{HH} = 6.0$ Hz, 4H, CH_2COCH_2), 3.75 (s, 2H, $N_{Pip}CH_2C_{Traiz}$), 4.00–4.10 (m, 4H, $POCH_2$), 4.43 (t, $^3J_{HH} = 7.0$ Hz, 2H, $NC\underline{H}_2CH_2CH_2P$), 7.53 (s, 1H, $C_{Traiz}H$). ^{13}C NMR ($CDCl_3$, 100.61 MHz), δ: 15.67 (d, $^3J_{CP} = 6.0$ Hz, $POCH_2\underline{C}H_3$), 21.66 (d, $^1J_{CP} = 143.5$ Hz, CH_2P), 22.90 (d, $^2J_{CP} = 4.5$ Hz, $NCH_2\underline{C}H_2CH_2P$), 40.27 ($\underline{C}H_2CO\underline{C}H_2$), 49.13 (d, $^3J_{CP} = 15.9$ Hz, $N\underline{C}H_2CH_2CH_2P$), 51.46 ($N_{Traiz}CH_2N_{Pip}$), 51.88 ($NC_{Pip}H_2$), 60.97 (d, $^2J_{CP} = 6.8$ Hz, $POCH_2$), 122.36 ($C_{Traiz}H$), 143.45 (C_{Traiz}), 207.72 (C=O). Anal. calcd. for $C_{15}H_{27}N_4O_4P{\cdot}0.25CHCl_3$ (%): C, 47.18; H, 7.07; N, 14.43; P, 7.98. Found (%): C, 47.07; H, 7.07; N, 14.52; P, 8.02.

*Diethyl 4-{4-[(4-oxopiperidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}butylphosphonate (**5**)*

Light yellow oil (65 %). ^{31}P NMR ($CDCl_3$, 161.97 MHz), δ: 30.88. ^{1}H NMR ($CDCl_3$, 400.13 MHz), δ: 1.27 (t, $^3J_{HH} = 7.1$ Hz, 6H, $POCH_2C\underline{H}_3$), 1.57–1.77 (m, 4H, $NCH_2CH_2C\underline{H}_2C\underline{H}_2P$), 2.01 (m, 2H, $NCH_2C\underline{H}_2CH_2CH_2P$), 2.40 (t, $^3J_{HH} = 6.0$ Hz, 4H, $NC_{Pip}H_2$), 2.78 (t, $^3J_{HH} = 6.0$ Hz, 4H, CH_2COCH_2), 3.75 (s, 2H, $N_{Pip}CH_2N_{Traiz}$), 3.98–4.09 (m, 4H, $POCH_2$), 4.34 (t, $^3J_{HH} = 7.1$ Hz, 2H, $NC\underline{H}_2CH_2CH_2CH_2P$), 7.48 (s, 1 H, $C_{Traiz}H$). Anal. calcd. for $C_{16}H_{29}N_4O_4P$ (%): C, 51.60; H, 7.85; N, 15.04; P, 8.32. Found (%): C, 51.69; H, 7.91; N, 14.99; P, 8.36.

*General procedure for the synthesis of phosphonates **1** and **2***

Boron trifluoride etherate (7 ml) was added to piperidone **4** or **5** (2 mmol) at 0 °C (ice bath). Then corresponding aldehyde (4 mmol) was added and the reaction mixture was magnetically stirred at room temperature for 6–7 days (the reaction course was monitored using TLC technique). Chloroform and aqueous solution of $NaHCO_3$ were added to the reaction mixture. Organic phase was separated; aqueous layer was extracted with chloroform. Combined organic solution was dried over anhydrous sodium sulfate, filtered, and evaporated at reduced pressure leaving yellow product as solid or sticky substance, which solidified after being left under layer of hexane. Purification using silica gel column chromatography (column: *l*, 27 cm, *d*, 2 cm; eluent: gradient from CH_2Cl_2 to CH_2Cl_2/MeOH = 100:2) and additional recrystallization, if needed, gave desired product as light yellow solid. Details for purification procedure and yields are given below for each compound separately.

*Diethyl 3-(4-{[(3E,5E)-3,5-bis(4-nitrobenzylidene)-4-oxo-1-piperidinyl]methyl}-1H-1,2,3-triazol-1-yl)propylphosphonate (**1a**)*

Purification by chromatography followed by recrystallization from a mixture of benzene/hexane. Yellow crystalline substance (0.56 g, 45 %), m.p. 127 °C (decomp.). IR (KBr), ν/cm^{-1}: 1673 (C=O), 1614 (C=C), 1597, 1519 (NO_2), 1343 (NO_2), 1247 (P=O), 1218, 1188, 1052, 1027 (P–O–C), 1000, 962, 859, 808, 759, 714, 687, 518. ^{31}P NMR ($CDCl_3$, 121.49 MHz), δ: 29.90. ^{1}H NMR ($CDCl_3$, 300.13 MHz), δ: 1.28 (t, $^3J_{HH} = 7.0$ Hz, 6H, $POCH_2C\underline{H}_3$), 1.63 (dt, $^3J_{HH} = 7.7$ Hz, $^2J_{PH} = 18.6$ Hz, 2H, $NCH_2CH_2C\underline{H}_2P$), 2.10–2.20 (m, 2H, $NCH_2C\underline{H}_2CH_2P$), 3.88 (s, 2H, $N_{Pip}CH_2C_{Traiz}$), 3.90 (s, 4H, $NC_{Pip}H_2$), 4.01–4.09 (m, 4H, $POCH_2$), 4.39 (t, $^3J_{HH} = 7.0$ Hz, 2H, $NC\underline{H}_2CH_2CH_2P$), 7.48 (s, 1H, $C_{Traiz}H$), 7.51 (d, $^3J_{HH} = 8.7$ Hz, 4H, *m*-$C_{Ar}H$ to NO_2), 7.78 (s, 2H, C_{Ar}–CH=), 8.26 (d, $^3J_{HH} = 8.7$ Hz, 4H, *o*-$C_{Ar}H$ to NO_2). ^{13}C NMR ($CDCl_3$, 100.61 MHz), δ: 16.22 (d, $^3J_{CP} = 5.9$ Hz, $POCH_2\underline{C}H_3$), 22.20 (d, $^1J_{CP} = 143$ Hz, CH_2P), 23.39 (d, $^2J_{CP} = 4.8$ Hz, $NCH_2\underline{C}H_2CH_2P$), 49.71 (d, $^3J_{CP} = 14.4$, $N\underline{C}H_2CH_2CH_2P$), 52.00 ($N_{Pip}CH_2C_{Traiz}$), 54.02 ($NC_{Pip}H_2$), 61.62 (d, $^2J_{CP} = 6.7$ Hz, $POCH_2$), 122.92 ($C_{Traiz}H$), 123.65 (*o*-C_{Ar} to NO_2), 130.68 (*m*-C_{Ar} to NO_2), 133.95 (=$\underline{C}$H–C_{Ar}), 135.46 ($\underline{C}$=CH–C_{Ar}), 140.96 (*p*-C_{Ar} to

NO_2), 143.14 (C_{Traiz}), 147.33 (C_{Ar}–NO_2), 186.06 (C=O). Anal. calcd. for $C_{29}H_{33}N_6O_8P$ (%): C, 55.77; H, 5.33; N, 13.46. Found (%): C, 55.81; H, 5.18; N, 13.54.

*Diethyl 3-(4-{[(3E,5E)-3,5-bis(4-cyanobenzylidene)-4-oxo-1-piperidinyl]methyl}-1H-1,2,3-triazol-1-yl)propylphosphonate (**1b**)*

Purification by chromatography followed by recrystallization from a mixture of EtOAc/hexane. Yellow crystalline substance (0.58 g, 50 %), m.p. 144 °C (decomp.). IR (KBr), ν/cm^{-1}: 2226 (C≡N), 1673 (C=O), 1614 (C=C), 1602, 1584, 1502, 1460, 1443, 1414, 1391, 1329, 1310, 1290, 1251 (P=O), 1234, 1218, 1190, 1176, 1163, 1096, 1081, 1050, 1028 (P–O–C), 999, 963, 930, 917, 861, 830, 801, 783, 758, 653, 627, 560. ^{31}P NMR ($CDCl_3$, 161.97 MHz), δ: 29.79. ^{1}H NMR ($CDCl_3$, 400.13 MHz), δ: 1.28 (t, $^3J_{HH}$ = 7.1 Hz, 6H, $POCH_2C\underline{H}_3$), 1.63 (dt, $^3J_{HH}$ = 7.7 Hz, $^2J_{PH}$ = 18.6 Hz, 2H, $NCH_2CH_2C\underline{H}_2P$), 2.09–2.20 (m, 2H, $NCH_2C\underline{H}_2CH_2P$), 3.86 (s, 6H, $NC_{Pip}H_2$ + $N_{Pip}CH_2C_{Traiz}$), 3.99–4.12 (m, 4H, $POCH_2$), 4.38 (t, $^3J_{HH}$ = 7.0 Hz, 2H, $NC\underline{H}_2CH_2CH_2P$), 7.44–7.46 (m, 5H, *m*-$C_{Ar}H$ to CN + $C_{Traiz}H$), 7.68–7.72 (m, 6H, *o*-$C_{Ar}H$ to CN + C_{Ar}–CH=). ^{13}C NMR ($CDCl_3$, 100.61 MHz), δ: 16.18 (d, $^3J_{CP}$ = 5.8 Hz, $POCH_2\underline{C}H_3$), 22.14 (d, $^1J_{CP}$ = 143 Hz, CH_2P), 23.33 (d, $^2J_{CP}$ = 5.1 Hz, $NCH_2\underline{C}H_2CH_2P$), 49.64 (d, $^3J_{CP}$ = 14.7 Hz, $N\underline{C}H_2CH_2CH_2P$), 51.95 ($N_{Pip}CH_2C_{Traiz}$), 53.97 ($NC_{Pip}H_2$), 61.57 (d, $^2J_{CP}$ = 7.0 Hz, $POCH_2$), 112.11 ($\underline{C}_{Ar}$–CN), 118.13 (CN), 122.85 ($C_{Traiz}H$), 130.38 (*m*-C_{Ar} to CN), 132.09 (*o*-C_{Ar} to CN), 134.26 (=$\underline{C}$H–C_{Ar}), 135.00 ($\underline{C}$=CH–C_{Ar}), 139.00 (*p*-C_{Ar} to CN), 143.17 (C_{Traiz}), 186.09 (C=O). Anal. calcd. for $C_{31}H_{33}N_6O_4P$ (%): C, 63.69; H, 5.69; N, 14.38. Found (%): C, 63.59; H, 5.54; N, 14.21.

*Diethyl 3-(4-{[(3E,5E)-3,5-bis(4-fluorobenzylidene)-4-oxo-1-piperidinyl]methyl}-1H-1,2,3-triazol-1-yl)propylphosphonate (**1c**)*

Purification by chromatography. Light-yellow crystalline substance (0.67 g, 59 %), m.p. 132–138 °C. IR (KBr), ν/cm^{-1}: 1673 (C=O), 1613 (C=C), 1600, 1580, 1508, 1471, 1303, 1294, 1285, 1245 (P=O), 1227, 1183, 1161, 1156, 1099, 1050, 1032 (P–O–C), 1026, 1013, 1000, 979, 958, 944, 926, 917, 849, 807, 786, 539, 496. ^{31}P NMR ($CDCl_3$, 161.97 MHz), δ: 30.02. ^{19}F NMR ($CDCl_3$, 281.40 MHz), δ: –110.43. ^{1}H NMR ($CDCl_3$, 400.13 MHz), δ: 1.27 (t, $^3J_{HH}$ = 7.0 Hz, 6H, $POCH_2C\underline{H}_3$), 1.62 (dt, $^3J_{HH}$ = 7.7 Hz, $^2J_{PH}$ = 18.6 Hz, 2H, $NCH_2CH_2C\underline{H}_2P$), 2.07–2.18 (m, 2H, $NCH_2C\underline{H}_2CH_2P$), 3.86 (s, 4H, $NC_{Pip}H_2$), 3.87 (s, 2H, $N_{Pip}CH_2C_{Traiz}$), 3.98–4.11 (m, 4H, $POCH_2$), 4.36 (t, $^3J_{HH}$ = 7.0 Hz, 2H, $N_{Traiz}C\underline{H}_2CH_2CH_2P$), 7.08 (t, $^3J_{HH}$ = $^3J_{FH}$ = 8.6 Hz, 4H, *o*-$C_{Ar}H$ to F), 7.34 (dd, $^3J_{HH}$ = 8.6 Hz, $^4J_{FH}$ = 6.0 Hz, 4H, *m*-$C_{Ar}H$ to F), 7.42 (s, 1H, $C_{Traiz}H$), 7.73 (s, 2H, C_{Ar}–CH=). ^{13}C NMR ($CDCl_3$, 100.61 MHz), δ: 16.20 (d, $^3J_{PC}$ = 5.7 Hz, $POCH_2\underline{C}H_3$), 22.20 (d, $^1J_{CP}$ = 143.3 Hz, CH_2P), 23.38 (d, $^2J_{CP}$ = 4.8 Hz, $NCH_2\underline{C}H_2CH_2P$), 49.68 (d, $^3J_{CP}$ = 14.9 Hz, $N\underline{C}H_2CH_2CH_2P$), 52.22 ($N_{Traiz}CH_2N_{Pip}$), 54.22 ($NC_{Pip}H_2$), 61.55 (d, $^2J_{CP}$ = 6.7 Hz, $POCH_2$), 115.59 (d, $^2J_{CF}$ = 21.6 Hz, *o*-C_{Ar} to F), 122.71 ($C_{Traiz}H$), 130.96 (d, $^4J_{CF}$ = 3.5 Hz, *p*-C_{Ar} to F), 132.16 (d, $^3J_{CF}$ = 8.6 Hz, *m*-C_{Ar} to F), 132.37 (d, $^6J_{CF}$ = 1.3 Hz, $\underline{C}$=CH–C_{Ar}), 135.14 (=$\underline{C}$H–C_{Ar}), 143.84 (C_{Traiz}), 162.69 (d, $^1J_{CF}$ = 251 Hz, C_{Ar}–F), 186.68 (C=O). Anal. calcd. for $C_{29}H_{33}F_2N_4O_4P$ (%): C, 61.05; H, 5.83; N, 9.82. Found (%): C, 61.07; H, 5.98; N, 9.82.

*Diethyl 3-(4-{[(3E,5E)-3,5-bis(4-methoxybenzylidene)-4-oxo-1-piperidinyl]methyl}-1H-1,2,3-triazol-1-yl)propylphosphonate (**1d**)*

Purification by chromatography followed by recrystallization from a mixture of benzene/hexane. Yellow crystalline solid (0.65 g, 52 %), m.p. 105–112 °C. IR (KBr), ν/cm^{-1}: 1668 (C=O), 1599 (C=C), 1567, 1510, 1460, 1315, 1304, 1291, 1255 (P=O), 1220, 1196, 1172, 1120, 1078, 1048, 1029 (P–O–C), 999, 963, 951, 931, 834, 789, 529. ^{31}P NMR ($CDCl_3$, 161.97 MHz), δ: 29.92. ^{1}H NMR ($CDCl_3$, 400.13 MHz), δ: 1.26 (t, $^3J_{HH}$ = 7.1 Hz, 6H, $POCH_2C\underline{H}_3$), 1.62 (dt, $^3J_{HH}$ = 7.7 Hz, $^2J_{PH}$ = 18.6 Hz, 2H, $NCH_2CH_2C\underline{H}_2P$), 2.05–2.18 (m, 2H, $NCH_2C\underline{H}_2CH_2P$), 3.82 (s, 6H, OCH_3), 3.87–3.88 (m, 6H, $NC_{Pip}H_2$ + $N_{Pip}CH_2C_{Traiz}$), 4.00–4.08 (m, 4H, $POCH_2$), 4.34 (t, $^3J_{HH}$ = 7.0 Hz, 2H, $NC\underline{H}_2CH_2CH_2P$), 6.92 (d, $^3J_{HH}$ = 8.8 Hz, 4H, *o*-$C_{Ar}H$ to MeO), 7.33 (d, $^3J_{HH}$ = 8.8 Hz, 4H, *m*-$C_{Ar}H$ to MeO), 7.39 (s, 1H, $C_{Traiz}H$), 7.73 (s, 2H, C_{Ar}–CH=). ^{13}C NMR ($CDCl_3$, 100.61 MHz), δ: 16.17 (d, $^3J_{CP}$ = 5.8 Hz, $POCH_2\underline{C}H_3$), 22.19 (d, $^1J_{CP}$ = 143.0 Hz, CH_2P), 23.33 (d, $^2J_{CP}$ = 4.5 Hz, $NCH_2\underline{C}H_2CH_2P$), 49.64 (d, $^3J_{CP}$ = 14.9 Hz, $N\underline{C}H_2CH_2CH_2P$), 52.28 ($N_{Traiz}CH_2N_{Pip}$), 54.43 ($NC_{Pip}H_2$), 55.07 (OMe), 61.49 (d, $^2J_{CP}$ = 6.8 Hz, $POCH_2$), 113.90 (*o*-C_{Ar} to OMe), 122.67 ($C_{Traiz}H$), 127.58 (*p*-C_{Ar} to OMe), 130.95 ($\underline{C}$=CH–C_{Ar}), 132.09 (*m*-C_{Ar} to OMe), 135.74 (=$\underline{C}$H–C_{Ar}), 144.16 (C_{Traiz}), 160.04 (C_{Ar}–OMe), 186.84 (C=O). Anal. calcd. for $C_{31}H_{39}N_4O_6P$: C, 62.62; H, 6.61; N, 9.42. Found (%): C, 62.81; H, 6.69; N, 9.39.

*Diethyl 4-(4-{[(3E,5E)-3,5-bis(4-nitrobenzylidene)-4-oxo-1-piperidinyl]methyl}-1H-1,2,3-triazol-1-yl)butylphosphonate (**2a**)*

Purification by chromatography followed by recrystallization from a mixture of benzene/hexane. Yellow crystalline solid (0.62 g, 49 %), m.p. 125 °C (decomp.). IR (KBr), ν/cm^{-1}: 1675 (C=O), 1649 (C=C), 1244 (P=O), 1052, 1026 (P–O–C). ^{31}P NMR ($CDCl_3$, 161.97 MHz), δ: 30.81. ^{1}H NMR ($CDCl_3$, 400.13 MHz), δ: 1.27 (t, $^3J_{HH}$ = 7.0 Hz,

6H, $POCH_2C\underline{H}_3$), 1.51–1.74 (m, 4H, $NCH_2CH_2C\underline{H}_2C\underline{H}_2P$), 1.90–1.98 (m, 2H, $NCH_2C\underline{H}_2CH_2CH_2P$), 3.87 (s, 2H, $N_{Pip}CH_2C_{Traiz}$), 3.89 (s, 4H, $NC_{Pip}H_2$), 3.99–4.10 (m, 4H, $POCH_2$), 4.28 (t, $^3J_{HH} = 7.1$ Hz, 2H, $N_{Traiz}CH_2$), 7.41 (s, 1H, $C_{Traiz}H$), 7.51 (d, $^3J_{HH} = 8.7$ Hz, 4H, *m*-$C_{Ar}H$ to NO_2), 7.78 (s, 2H, C_{Ar}–CH=), 8.26 (d, $^3J_{HH} = 8.7$ Hz, 4H, *o*-$C_{Ar}H$ to NO_2). ^{13}C NMR ($CDCl_3$, 75.47 MHz), δ: 16.25 (d, $^3J_{CP} = 5.5$ Hz, $POCH_2\underline{C}H_3$), 19.37 (d, $^2J_{CP} = 4.8$ Hz, $\underline{C}H_2CH_2P$), 24.55 (d, $^1J_{CP} = 142.0$ Hz, CH_2P), 30.44 (d, $^3J_{CP} = 15.2$ Hz, $\underline{C}H_2CH_2CH_2P$), 49.49 ($N\underline{C}H_2CH_2CH_2CH_2P$), 52.03 ($N_{Triaz}CH_2N_{Pip}$), 54.02 ($NC_{Pip}H_2$), 61.42 (d, $^2J_{CP} = 6.9$ Hz, $POCH_2$), 122.54 ($C_{Traiz}H$), 123.65 (*o*-C_{Ar} to NO_2), 130.71 (*m*-C_{Ar} to NO_2), 133.96 (=$\underline{C}$H–C_{Ar}), 135.49 ($\underline{C}$=CH–C_{Ar}), 140.99 (*p*-C_{Ar} to NO_2), 143.17 (C_{Traiz}), 147.33 (C_{Ar}–NO_2), 186.11 (C=O). Anal. calcd. for $C_{30}H_{35}N_6O_8P$ (%): C, 56.42; H, 5.52; N, 13.16. Found (%): C, 56.21; H, 5.54; N, 12.94.

*Diethyl 4-(4-{[(3E,5E)-3,5-bis(4-cyanobenzylidene)-4-oxo-1-piperidinyl]methyl}-1H-1,2,3-triazol-1-yl)butylphosphonate (**2b**)*

Purification by chromatography followed by precipitation from solution in chloroform with pentane. Yellow crystalline solid (0.71 g, 61 %), m.p. 145 °C (decomp.). IR (KBr), ν/cm^{-1}: 1677 (C=O), 1647 (C=C), 1240 (P=O), 1098, 1053, 1024 (P–O–C). ^{31}P NMR ($CDCl_3$, 161.97 MHz), δ: 30.73. ^1H NMR ($CDCl_3$, 400.13 MHz), δ: 1.28 (t, $^3J_{HH} = 7.0$ Hz, 6H, $POCH_2C\underline{H}_3$), 1.55–1.76 (m, 4H, $NCH_2CH_2C\underline{H}_2C\underline{H}_2P$), 1.91–1.98 (m, 2H, $NCH_2C\underline{H}_2CH_2CH_2P$), 3.86 (s, 6H, $NC_{Pip}H_2 + N_{Pip}CH_2C_{Traiz}$), 3.99–4.10 (m, 4H, $POCH_2$), 4.28 (t, $^3J_{HH} = 7.0$ Hz, 2H, $NC\underline{H}_2CH_2CH_2CH_2P$), 7.39 (s, 1H, $C_{Traiz}H$), 7.45 (d, $^3J_{HH} = 8.0$ Hz, 4H, *m*-$C_{Ar}H$ to CN), 7.68 (s, 2H, C_{Ar}–CH=), 7.71 (d, $^3J_{HH} = 8.0$ Hz, 4H, *o*-$C_{Ar}H$ to CN). ^{13}C NMR ($CDCl_3$, 100.61 MHz), δ: 16.20 (d, $^3J_{CP} = 5.8$ Hz, $POCH_2\underline{C}H_3$), 19.33 (d, $^2J_{CP} = 4.6$ Hz, $\underline{C}H_2CH_2P$), 24.54 (d, $^1J_{CP} = 142.0$ Hz, CH_2P), 30.37 (d, $^3J_{CP} = 14.6$ Hz, $NCH_2\underline{C}H_2CH_2CH_2P$), 49.43 ($N\underline{C}H_2CH_2CH_2CH_2P$), 52.02 ($N_{Triaz}CH_2N_{Pip}$), 54.00 ($NC_{Pip}H_2$), 61.35 (d, $^2J_{CP} = 6.3$ Hz, $POCH_2$), 112.13 ($\underline{C}_{Ar}$–CN), 118.14 (CN), 122.41 ($C_{Traiz}H$), 130.40 (*m*-C_{Ar} to CN), 132.09 (*o*-C_{Ar} to CN), 134.27 (=$\underline{C}$H–C_{Ar}), 135.02 ($\underline{C}$=CH–C_{Ar}), 139.02 (*p*-C_{Ar} to CN), 143.23 (C_{Traiz}), 186.13 (C=O). Anal. calcd. for $C_{32}H_{35}N_6O_4P$ (%): C, 64.20; H, 5.89; N, 14.04. Found (%): C, 64.00; H, 5.58; N, 13.80.

*General procedure for the synthesis of phosphonic acids **6a**, **b***

A solution of trimethylbromosilane (3.6 mmol) in chloroform (2 ml) was added to solution of corresponding phosphonate (0.3 mmol) in anhydrous chloroform (3 ml). The reaction solution obtained was left to stay at room temperature in closed flask in dark place for two weeks. Then it was evaporated to dryness at reduced pressure, the product remained was treated with 8 ml of MeOH, and then with water until complete precipitation of acid was observed. The desired compound was filtered, washed with water, and dried on air to give corresponding acid as yellow powder.

*[3-(4-{[(3E,5E)-3,5-bis(4-nitrobenzylidene)-4-oxopiperidin-1-yl]methyl}-1H-1,2,3-triazol-1-yl)propyl]phosphonic acid (**6a**)*

Yellow powder substance (84 %), m.p. 185 °C (decomp.). ^{31}P NMR (DMSO-d_6, 161.97 MHz), δ: 24.92. ^1H NMR (DMSO-d_6, 400.13 MHz), δ: 1.40–1.49 (m, 2H, $NCH_2CH_2C\underline{H}_2P$), 1.97 (m, 2H, $NCH_2C\underline{H}_2CH_2P$), 4.44 (t, $^3J_{HH} = 6.2$ Hz, 2H, $NC\underline{H}_2CH_2CH_2P$), 4.59 (s, 2H, $N_{Pip}CH_2C_{Traiz}$), 4.64 (s, 4H, $NC_{Pip}H_2$) 7.79 (d, $^3J_{HH} = 8.3$ Hz, 4H, *m*-$C_{Ar}H$ to NO_2), 7.97 (s, 2H, CH=), 8.30 (s, 1H, $C_{Traiz}H$), 8.36 (d, $^3J_{HH} = 8.3$ Hz, 4H, *o*-$C_{Ar}H$ to NO_2). Anal. calcd. for $C_{25}H_{25}N_6O_8P{\cdot}0.33H_2O{\cdot}0.92HBr$ (%): C, 46.29; H, 4.13; N, 12.96; Br, 11.29. Found (%): C, 46.27; H, 4.00; N, 12.74; Br, 11.49.

*[3-(4-{[(3E,5E)-3,5-bis(4-cyanobenzylidene)-4-oxopiperidin-1-yl]methyl}-1H-1,2,3-triazol-1-yl)propyl]phosphonic acid (**6b**)*

Yellow powder substance (85 %), m.p. 175 °C (decomp.). ^{31}P NMR (DMSO-d_6, 161.97 MHz), δ: 25.12. ^1H NMR (DMSO-d_6, 400.13 MHz), δ: 1.44–1.53 (m, 2H, $NCH_2CH_2C\underline{H}_2P$), 1.92–2.03 (m, 2H, $NCH_2C\underline{H}_2CH_2P$), 4.47 (t, $^3J_{HH} = 6.7$ Hz, 2H, $NC\underline{H}_2CH_2CH_2P$), 4.71 (s, 2H, $N_{Pip}CH_2C_{Traiz}$), 4.75 (s, 4H, $NC_{Pip}H_2$) 7.71 (d, $^3J_{HH} = 8.3$ Hz, 4H, *m*-$C_{Ar}H$ to CN), 7.95 (s, 2H, CH=), 8.01 (d, $^3J_{HH} = 8.3$ Hz, 4H, *o*-$C_{Ar}H$ to CN), 8.34 (s, 1H, $C_{Traiz}H$). ^{13}C NMR (DMSO-d_6, 100.61 MHz), δ: 24.18 (d, $^2J_{CP} = 3.1$ Hz, $NCH_2\underline{C}H_2CH_2P$), 24.36 (d, $^1J_{CP} = 138.0$ Hz, CH_2P), 49.26 ($N_{Pip}CH_2C_{Traiz}$), 49.92 (d, $^3J_{CP} = 17.0$ Hz, $N\underline{C}H_2CH_2CH_2P$), 50.98 ($NC_{Pip}H_2$), 112.24 ($\underline{C}_{Ar}$–CN), 118.55 (CN), 127.46 ($C_{Traiz}H$), 129.32 (=$\underline{C}$H–C_{Ar}), 131.20 (*m*-C_{Ar} to CN), 132.81 (*o*-C_{Ar} to CN), 136.03 (C=CH–C_{Ar}), 138.01 (C_{Traiz}), 138.39 (*p*-C_{Ar} to CN), 181.38 (C=O). Anal. calcd. for $C_{27}H_{25}N_6O_4P{\cdot}H_2O{\cdot}1.5HBr$ (%): C, 48.56; H, 4.30; N, 12.58; Br, 17.95. Found (%): C, 48.76; H, 4.03; N, 12.88; Br, 17.59.

X-ray structure determination

The single crystals of **1c** suitable for X-ray experiments were obtained by slow diffusion of pentane into a chloroform solution of the compound. Data were collected on a Bruker APEX-II CCD diffractometer ((MoK_α)-radiation, graphite monochromator, φ and ω scan mode), and corrected for absorption using the *SADABS* program v. 2.03

Table 3 Crystallographic Data for **1c**

Empirical formula	$C_{29}H_{33}N_4O_4F_2P$
Fw	570.56
T, K	100(2)
Crystal system	Triclinic
Space group	P-1
a, Å	5.641 (2)
b, Å	15.295 (7)
c, Å	16.962 (7)
α, deg.	68.864 (8)
β, deg.	81.039 (9)
γ, deg.	86.965 (9)
V, Å^3	1,348.3 (10)
Z	2
d_c, g cm^{-3}	1.405
$F(000)$	600
μ, mm^{-1}	0.160
$2\theta_{max}$, deg.	50
Reflections collected/unique/R_{int}	11,326/4,611/0.072
Reflections with $I > 2\sigma(I)$	2,823
R_1 ($I > 2\sigma(I)$)	0.095
wR_2 (all data)	0.242
GOF on F^2	1.000

(Bruker/Siemens Area detector absorption correction program). For details, see Table 3. The structure was solved by direct methods and refined by a full-matrix least squares technique on F^2 with anisotropic displacement parameters for non-hydrogen atoms. One of the two ethoxy groups of the phosphonate fragment is disordered over two sites with the equal occupancies. All hydrogen atoms were placed in calculated positions and refined within the riding model with fixed isotropic displacement parameters ($U_{iso}(H) = 1.5Ueq(C)$ 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 (Sheldrick, 2008). Crystallographic data for **1c** have been deposited with the Cambridge Crystallographic Data Center. CCDC 955385 contains the 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; 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), CaoV3 (ovarian cancer), A549 (lung cancer), PC3 (prostate cancer), as well as HEF. 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 the concentration of 1×10^4 cells/100 ml/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 ml 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 (OD) 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 was used as a positive control in the assay.

Acknowledgments This work was supported by the Deutsche Forschungsgemeinschaft (project no. RO 362/51/1) and the Russian Foundation for Basic Research (project no. 14-03-00687).

References

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

Artyushin OI, Vorob'eva DV, Vasileva TV, Osipov SN, Röschenthaler GV, Odinets IL (2008) Facile synthesis of phosphorylated azides in ionic liquids and their use in the preparation of 1,2,3-trazoles. Heteroatom Chem 19:293–300

Artyushin OI, Osipov SN, Röschenthaler GV, Odinets IL (2009) Propargyl-substituted phosphonocarboxylates: efficient synthesis and application to click chemistry. Synthesis 21:3579–3588

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 (2009) 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, Sakagami H, Hashimoto K, Kawase M, Gorecki DK, Dimmock JR (2010) Sequential cytotoxicity: a theory examined using a series of 3,5-bis(benzylidene)-1-diethylphosphono-4-oxopiperidines and related phosphonic acids. Bioorg Med Chem Lett 20:6464–6468

El-Mabhouh AA, Mercer JR (2008) ^{188}Re-labelled gemcitabine/bisphosphonate (Gem/BP): a multi-functional, bone-specific agent as a potential treatment for bone metastases. Eur J Nucl Med Mol Imaging 35:1240–1248

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

Fabulet O, Sturtz G (1995) Synthesis of gem-bisphosphonic doxorubicin conjugates. Phosphorus, Sulfur Silicon Relat Elem 101:225–234

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

Ledoux D, Hamma-Kourbali Y, Di Benedetto M, Foucault-Bertaud A, Oudar O, Sainte-Catherine O, Lecouvey M, Kraemer M (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 ES, Makarov MV, Rybalkina EYu, Nayani SL, Tongwa P, Fonari A, Timofeeva TV, Odinets IL (2010a) Structure–cytotoxicity relationship in a series of N-phosphorus substituted E, E-3,5-bis(3-pyridinylmethylene)- and E, E-3,5-bis(4-pyridinylmethylene)piperid-4-ones. Eur J Med Chem 45:5926–5934

Leonova E, Makarov M, Klemenkova Z, Odinets I (2010b) 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, Tongwa P, Khrustalev VN, Timofeeva TV, Odinets IL (2010) Synthesis, characterization and structure–activity relationship of novel N-phosphorylated E, E-3,5-bis(thienylidene)piperid-4-ones. Eur J Med Chem 45:992–1000

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

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

Odinets IL, Artyushin OI, Goryunov EI, Lyssenko KA, Rybalkina EYu, Kosilkin IV, Timofeeva TV, Antipin MYu (2005) N-Phosphorylated 3,5-bis(arylidene)-4-piperidones: synthesis, X-ray structure, and evaluation of antitumor activity. Heteroat Chem 16:497–502

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, New York, pp 427–475

Reinholz MM, Zinnen SP, Dueck AC, Dingli D, Reinholz GG, Jonart LA, Kitzmann KA, Bruzek AK, Negron V, Abdalla AK, Arendt BK, Croatt AJ, Sanchez-Perez L, Sebesta DP, Lönnberg H, Yoneda T, Nath KA, Jelinek DF, Russell SJ, Ingle JN, Spelsberg TC, Hal Dixon BF, Karpeisky A, Lingle WL (2010) A promising approach for treatment of tumor-induced bone diseases: utilizing bisphosphonate derivatives of nucleoside antimetabolites. Bone 47:12–22

Sheldrick GM (2008) A short history of SHELX. Acta Cryst A64:112–122

Shipov AE, Makarov MV, Petrovskii PV, Rybalkina EYu, Nelyubina YuV, Odinets IL (2013) 3,5-Bis(arylidene)piperid-4-ones containing 1,3,2-oxazaphosphorinane moieties: synthesis and antitumor activity. Heteroatom Chem 24:191–199

Skarpos H, Osipov SN, Vorob'eva DV, Odinets IL, Lork E, Röschenthaler GV (2007) Synthesis of functionalized bisphosphonates via click chemistry. Org Biomol Chem 5:2361–2367

Sturtz G, Couthon H, Fabulet O, Mian M, Rosini S (1993) Synthesis of gem-bisphosphonic methotrexate conjugates and their biological response towards Walker's osteosarcoma. Eur J Med Chem 28:899–903

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