European Journal of Medicinal Chemistry 45 (2010) 5926–5934

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

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

Evgeniya S. Leonova [a], Michael V. Makarov [a], Ekaterina Yu. Rybalkina [b], Shravana L. Nayani [c], Paul Tongwa [c], Alexander Fonari [c], Tatiana V. Timofeeva [c], Irina L. Odinets [a,*]

[a] A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Vavilova str., 28, 119991 Moscow, Russian Federation
[b] Institute of Carcinogenesis, N.N. Blokhin Russian Cancer Research Centre, Russian Academy of Medical Sciences, Kashirskoe sh., 24, 115478 Moscow, Russian Federation
[c] New Mexico Highlands University, Las Vegas, NM 87701, USA

ARTICLE INFO

Article history:
Received 28 July 2010
Received in revised form
23 September 2010
Accepted 27 September 2010
Available online 28 October 2010

Keywords:
3,5-bis((hetero)arylidene)piperid-4-ones
N-Phosphoryl-3,5-bis(pyridinylmethylene) piperid-4-ones
4-Piperidones
N-Phosphorylalkylene-3,5-bis (pyridinylmethylene)piperid-4-ones
Synthesis
X-ray structure
Cytotoxicity–structure relationship

ABSTRACT

In order to give further insight on the influence of the aromatic ring nature and the presence of the phosphorus substituent at the piperidone nitrogen atom of *E*,*E*-3,5-bis((hetero)arylidene)piperid-4-ones on their antitumor properties, a series of phosphorus substituted *E*,*E*-3,5-bis(pyridinylmethylene) piperid-4-ones bearing either 3-pyridine or 4-pyridine rings was obtained. Novel NH-3,5-bis(pyridinylmethylene)piperid-4-ones **1a**,**b** were converted into the corresponding N-phosphorylated derivatives **3a**–**c**, **4a**–**c** differing in the substitution at the phosphorus atom (amidophosphates and amidophosphonates), via direct phosphorylation while N-(ω-phosphorylalkyl)-substituted compounds **8a**–**c** were obtained via aldol-crotonic condensation of preformed N-phosphorylalkyl substituted piperidones with the corresponding pyridinecarboxaldehyde. The cytotoxicity screen has revealed that phosphorylated compounds based on *E*,*E*-3,5-bis(4-pyridinylmethylene)piperid-4-one framework displayed higher inhibitory properties toward Caov3, A549, KB 3-1 and KB 8-5 human carcinoma cell lines comparing with their analogues with 3-pyridine rings. Introduction of the phosphorus moiety substantially increased the antitumor properties in the case of *E*,*E*-3,5-bis(3-pyridinylmethylene)piperid-4-ones derivatives but this influence less pronounced for more active analogues bearing 4-pyridinyl rings. Most of the compounds tested are potent against multi-drug resistant cell line KB 8-5 affording some guidelines for the search of perspective drug-candidates among phosphorus substituted *E*,*E*-3,5-bis ((hetero)arylidene)piperid-4-ones.

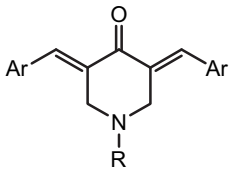
1. Introduction

Starting from early 1970s bis(arylidene)piperid-4-ones and the related bis(arylidene)cycloalkan-4-ones were intensively studied in relation to their cytotoxic properties and antitumor activities [1–7]. The excellent cytotoxic properties of different compounds possessing the general structure **I** is believed to be attributed to the presence of 1,5-diaryl-3-oxo-1,4-pentadienyl (dienone) pharmacophore moiety observed both in alicyclic unsaturated enones and cyclic piperidone derivatives [4]. The proposed mechanism of action of such compounds includes their interaction with biogenic thiols (i.e. their alkylation), the nitrogen-containing intracellular nucleophiles such as proteins and nucleic acids not being affected by them [8,9]. This fact allows to consider 3,5-bis(arylidene)-piperid-4-ones as potential drug-candidates which may be free of mutagenic properties of known alkylating anticancer agents used in current medicine practice [10]. Moreover, these compounds (generally screened as free bases) are non-lethal and mice tolerated doses up to 300 mg/kg [11] and even five daily doses up to 240 mg/kg of hydrochloride salt of 3,5-bis(benzylidene)piperid-4-one did not cause mortalities [12].

Many investigations indicate that the presence of strong electron-withdrawing substituents in aryl moieties results in significant increase of antitumor activity of 3,5-bis(arylidene)-piperid-4-ones. In other words, a Hammett σ_P value of the substituents in the aryl rings (as well as conformation of the central core) and hence that of the substituted aryl ring affects the fractional positive change on the adjacent carbon atoms at the double bond and, as a consequence, the possibility of the compound to interact with cellular thiols [13]. As well known, σ_P value of 3-pyridinyl group is close to that for 4-NO_2–C_6H_4 (σ_P = 0.25 and 0.26, respectively) while the Hammett constant of

* Corresponding author. Tel.: +7 495 1359356; fax: +7 495 1359356.
E-mail address: odinets@ineos.ac.ru (I.L. Odinets).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.09.058



IA-D

A: Ar = substituted phenyl,
R = H, Alk, C(O)Alk, C(O)OAlk

B: Ar = 4-$C_6H_4NMe_2$,
R = $P(O)(OCH_2CF_3)_2$, $P(O)(OPh)_2$, P(O)(OPh)Me
Ar = 4-$C_6H_4NO_2$, 3-$C_6H_4NO_2$, 2-$C_6H_4NO_2$
R = $P(O)(OEt)_2$, $P(O)(OH)_2$

C: Ar = 4-$C_6H_4R^1$, R^1 = NMe_2, F, NO_2,
R = $(CH_2)_nP(O)(OEt)_2$ or $(CH_2)_nP(O)(ONa)_2$, n = 1-4

D: Ar = 2-thienyl,
R = $P(O)(OEt)_2$, $P(O)(OPh)_2$, P(O)(OEt)Me, or
$(CH_2)_nP(O)(OEt)_2$, (n = 1-3)

Scheme 1. Modified structures of 3,5-bis(arylidene)-piperid-4-ones.

4-pyridinyl is much higher ($\sigma_P = 0.44$) [14]. Therefore, one may expect high cytotoxicity for 3,5-bis(pyridinylmethylene)-piperid-4-ones. Nevertheless, only one compound belonging to pyridine substituted 3,5-bis(arylidene)-piperid-4-ones (namely N,N-dimethyl-3,5-bis (4-pyridinylmethylene)-4-piperidonium iodide) was tested for this type of activity [15] and reported to possess the diminished antitumor properties comparing with a wide range of 3,5-bis(arylidene)-piperid-4-ones in the screens used an average of 54 human tumor cell lines from eight neoplastic diseases such as leukemia, melanoma, colon, non-small-cell lung, small-cell lung, central nervous system, ovarian, and renal cancers.

On the other hand, there has been a reasonable suggestion that the groups at the piperidone nitrogen atom may influence the cytotoxic properties of 3,5-bis(arylidene)piperid-4-ones via their interactions with additional binding sites of a biological target. These groups can contribute to solubility and bioavailability of the final compound and increase the cytotoxic properties, for example, by facilitating approach of the cytotoxin to a specific binding site. However, the above groups can also reduce cytotoxicity preventing this interaction. Moreover, according to the hypothesis of sequential cytotoxicity, if these additional groups are also able to alkylate intracellular nucleophiles, the malignant cells may suffer greater injury than normal ones. That means that structure–activity relationship of compounds of such type presents a multifactor problem.

Therefore, a great number of publications dealing with modification of structures of 3,5-bis(arylidene)-piperid-4-ones in order to elucidate the structure–activity relationship, enhance antitumor activity and selectivity to different types of cancer, improve the capacity of their transportation via the cellular membrane and bioavailability, and to impart them such properties as ability to reverse multi-drug resistance can be found in the literature. Mostly, these modifications include variation of the substituents at the aryl rings, which differ in electronic, hydrophobic, and steric properties, and alkylation or acylation of the piperidone nitrogen atom of the ring (Scheme 1, compounds **A**) [4,5,16].

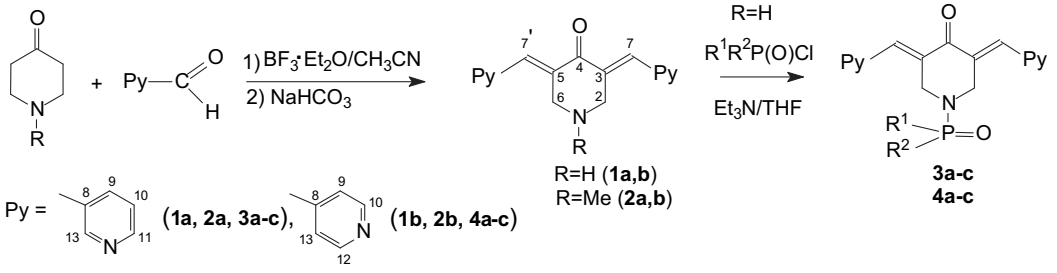
More recently, it was revealed that introduction of phosphonate (phosphinate) group [17–19] or ω-phosphonoalkyl moiety [19,20] at the piperidone nitrogen atom significantly increase cytotoxic properties of 3,5-bis(arylidene)-piperid-4-ones (compounds **B**–**C**, Scheme 1). This phenomenon was more pronounced in the case of 3,5-bis(2-thienylidene)-piperid-4-ones **D**. Thus, while NH-3,5-bis (2-thienylidene)-piperid-4-one and 1-methyl-3,5-bis(2-thienylidene)-piperid-4-one did not show significant cytotoxicity towards human carcinoma cell lines Scov3, Caov3 and A549 (IC_{50} values ≥ 80 μM), their phosphorylated analogues were more active (IC_{50} values in the range of 4–60 μM), with the methylphosphinate (R = P(O)(OEt)Me) having the lowest IC_{50} values among all investigated thiophene derivatives **D**. Furthermore, the above mentioned general tendency of more prominent cytotoxicity of derivates bearing electron-withdrawing substituents as compared to those having electron-donating ones was also observed for phosphorus-containing 3,5-bis(arylidene)-piperid-4-ones **B** and **C**.

In view of these results, in this paper we report on the synthesis, crystal structure, and antitumor activity *in vitro* of phosphoryl substituted 3,5-bis(pyridinylmethylene)-piperid-4-ones differing in the position of the nitrogen atom in pyridine ring of the dienone system.

2. Results and discussion

2.1. Chemistry

In order to give insight into the influence of the phosphorus moiety on cytotoxic properties of 3,5-bis(pyridinylmethylene) piperid-4-ones, we performed the synthesis of the corresponding 3-pyridinyl and 4-pyridinyl derivatives bearing either phosphorus atom in different surrounding directly bonded with the nitrogen atom of the piperidone cycle or those having an alkylene linker between the phosphorus and the nitrogen atoms.



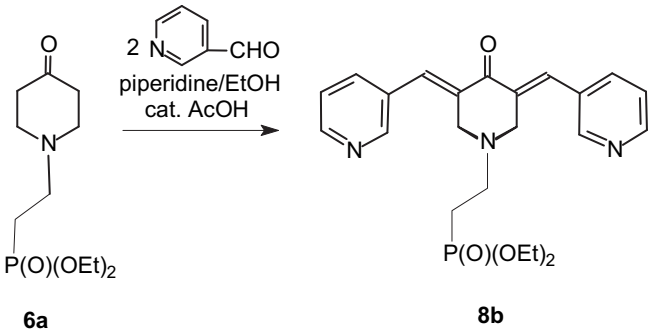
Scheme 2. Synthesis of 3,5-bis(pyridinylmethylene)piperid-4-ones **1a**,**b**,**2a**,**b** and phosphorylation of compounds **1a**,**b**.

For the synthesis of the first series of the target compounds the direct phosphorylation of the preformed parent NH-3,5-bis(pyridinylmethylene)piperid-4-ones **1a**,**b** by the appropriate phosphorus(V) acid chlorides in the presence of triethylamine as a base was used (Scheme 2).

The starting NH-precursors **1a**,**b** were obtained via the aldol-crotonic condensation of the corresponding pyridinecarboxaldehyde and piperid-4-one in the presence of boron trifluoride etherate (r.t., CH_3CN as a solvent) as the corresponding tris(tetrafluoroborate) salts followed by neutralization according to procedure elaborated by us recently [21]. The similar approach was used for the synthesis of known N-methyl-3,5-bis(pyridinylmethylene)piperid-4-ones **2a**,**b** [22] used for comparison in cytotoxicity screen (see below). Note, that the above aldol-crotonic condensation provided high yields in the reaction of NH-piperid-4-one with 3-pyridinecarboxaldehyde and those of N-methyl-piperid-4-one with both aldehydes (90% isolated yield) while the compound **1b**·HBF_4 was obtained in 20% yield only. Moreover, the condensation of 4-piperidone with 2-pyridinecarboxaldehyde afforded 1,2-di(2-pyridinyl)-1,2-ethane -dione along with traces of (3*E*)-3-(pyridin-2-ylmethylene)piperidin-4-one (monocondensation product) rather than the related NH-3,5-bis(2-pyridinylmethylene)piperid-4-one **1d**.

As regards the further phosphorylation of **1a**,**b**, it should be emphasized that the starting substrates **1a**,**b** require careful drying over P_2O_5 in vacuum for successful reaction as they are inclined to form stable hydrates. The presence of water results in domination of side reactions dealing with the hydrolysis of phosphorylating agent and further formation of pirophosphates (pirophosphonates) hence reducing significantly the yields of the desired amidophosphates (phosphonates). The higher yields of derivatives **3a**–**c** bearing 3-pyridinyl fragment comparing with the products **4a**–**c** over phosphorylation may be attributed to the lesser stability of hydrate formed by NH-3,5-bis(3-pyridinylmethylene)piperid-4-one **1a** which hence contained less amount of water after drying (the drying conditions were the same for both substrates).

As introduction of the ω-phosphorylalkyl group to the nitrogen atom of the preformed NH-precursors **1a**,**b** is accompanied by quaternization [16], the aldol-crotonic condensation of 3-pyridinecarboxaldehyde with preformed aminophosphonates **5**,**6a**,**b** was the method of choice for the synthesis of ω-aminophosphonates **8a**–**c** differing in the length of an alkylene linker. Firstly, the reactions were performed in the presence of $BF_3 \cdot Et_2O$ both as a catalyst and a solvent. Application of additional co-solvent such as CH_3CN decreased the reaction rate and accelerated side reactions (hydrolysis of ester groups). Note that the longer linker has the starting ω-aminophosphonate the higher was the reaction rate (ca. 1 week for α-aminophosphonate **5** versus about 3 h for δ-aminophosphonate **6b**). Similar to the above mentioned synthesis of 3,5-bis(pyridinylmethylene)piperid-4-ones **1a**,**b** and



6a **8b**

Scheme 4. Synthesis of N-(2-phosphorylethyl)-3,5-bis(3-pyridinyl'methylene)-4-piperidone **8b**.

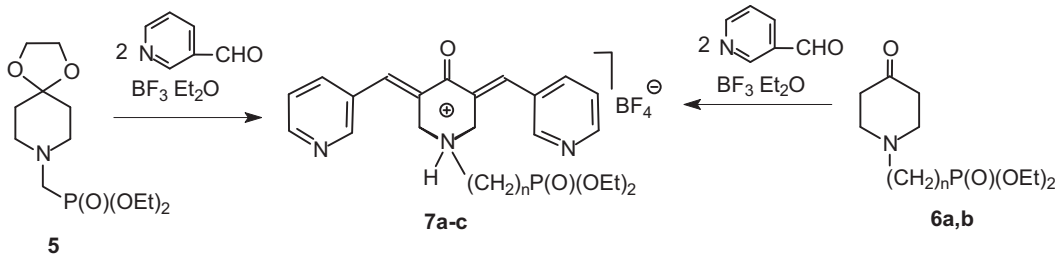
2a,**b**, the final tetrafluoroborate salts **7a**–**c** precipitated from reaction mixtures and were isolated via filtration (Scheme 3).

However, using further neutralization with aq. $NaHCO_3$ of tetrafluoroborate salts **7a**–**c** we succeeded to isolate in a pure form only N-(4-phosphorylbutyl)-3,5-bis(pyridinylmethylene)piperid-4-one **8c**, while we failed to purify free bases **8a**,**b** with shorter alkylene chains from unidentified side products using column chromatography and/or crystallization. Therefore, we performed the alternative synthesis of N-(2-phosphorylethyl)-substituted derivative **8b** via condensation of β-aminophosphonate **6a** with 3-pyridinecarboxaldehyde under basic conditions which resulted in **8b** in ca. 28% (Scheme 4).

The structures of the phosphorylated products were elucidated by ^1H, ^{31}P, ^{13}C NMR and IR spectral data along with a single crystal X-ray diffraction analysis. The ^{31}P NMR spectra of the corresponding products displayed the singlet signals observed at *ca.*7.2 ppm for amidophosphates **3a**,**4a** and at ca. 30 ppm for phosphonates **3b**,**c**, **4d**,**c**, and **8b**,**c**. Such disposition of the signals is typical for the mentioned surrounding at the particular phosphorus atom. In the ^1H NMR spectra of all compounds the singlet resonances assigned to vinyl hydrogen atoms are observed at ca. 8.0 ppm for tetrafluoroborate salts **7a**–**c** while in the spectra of the corresponding free bases **8b**,**c** these resonances are upfield schifted for ca. 7.7 ppm. The related tendency was observed in the corresponding pattern of the ^{13}C spectra: the signals of methyne C7(7′)-carbon atom observed at 143–146 ppm in the spectra tetrafluoroborate salts **7a**–**c** shifted up to 133–135 ppm in the spectra of the corresponding free bases **8b**,**c**.

2.2. Molecular and crystal structures of 3,5-bis (pyridinilmethylene)-4-piperidones

We succeeded to grow crystals of three derivatives **2a**,**3c** and **8b** that were satisfactory for a single crystal X-ray structural characterization. All compounds have 3-pyridyl rings and N-methyl, N-methyl(phenoxy)phosphoryl, and N-(2-diethoxyphosphoryl)ethyl



5 **7a-c** **6a,b**

n = 1 **(5, 7a)**, 2 **(6a, 7b)**, 3 **(6b, 7c)**

Scheme 3. Synthesis of N-(ω-phosphorylalkyl)-3,5-bis(3-pyridinylmethylene)-4-piperidone tetrafluoroborates **7a**–**c**.

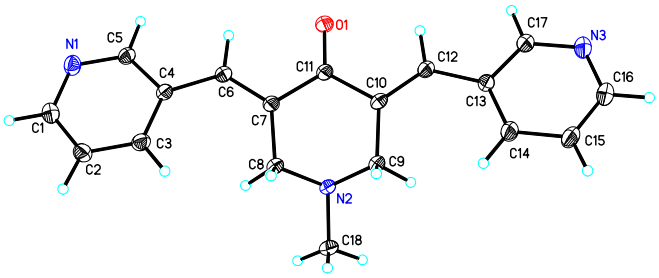


Fig. 1. Molecular structure of **2a** drawn at 50% probability of thermal ellipsoids.

groups, respectively. Structural features of molecules **2a**,**3c** and **8b** (Figs. 1–3) do not differ significantly from relative molecules that belong to groups **A**–**D** mentioned in introduction. Note that numeration of the atoms in the NMR spectra of the compounds and X-ray data is independent.

To the best of our knowledge, only one example of structural studies of pyridine substituted 3,5-bis(arylidene)-piperid-4-ones (namely N,N-dimethyl-3,5-bis(4-pyridinylmethylene)-4-piperidonium iodide) was presented in literature [15]. It was found that for this compound deviation of aryl cycles from the plane of central fragment does not exceed 30°. Studies of N-phosphorylated compounds have shown that introduction of even large substituents at the N atom of piperidone ring do not cause significant rotation of planes of phenyl or thiophene rings from the plane of central fragment [17,19]. However, for 3,5-bis(2-nitrobenzylidene)-4-oxo-1-phosphonopiperidine molecule having nitro group in the *ortho*-position, increase of the torsion angle around single bond between an aryl ring and adjusted unsaturated group, that can be explained by steric hindrances, was found [18].

In molecules of **2a**, **3c**, and **8b** under investigation two types of orientation of pyridine nitrogen atoms exist: towards carbonyl oxygen (**2a**, two nitrogens up) and towards piperidone nitrogen (**3c** and **8b**, two nitrogens down). This fact demonstrates that in the other crystalline samples and/or in solutions of 3,5-bis(hetero-arylidene)-piperid-4-ones up–up, down–down and up-down positions of heteroatom in heteroaromatic substituents can be expected, as it was found for thiophene-substituted molecules [19].

As in the previously studied molecules belonging to compounds of this class, the structures presented in this paper are characterized with three planar fragments shown on Scheme 5. Dihedral angles between these planes, mean atomic deviations from these

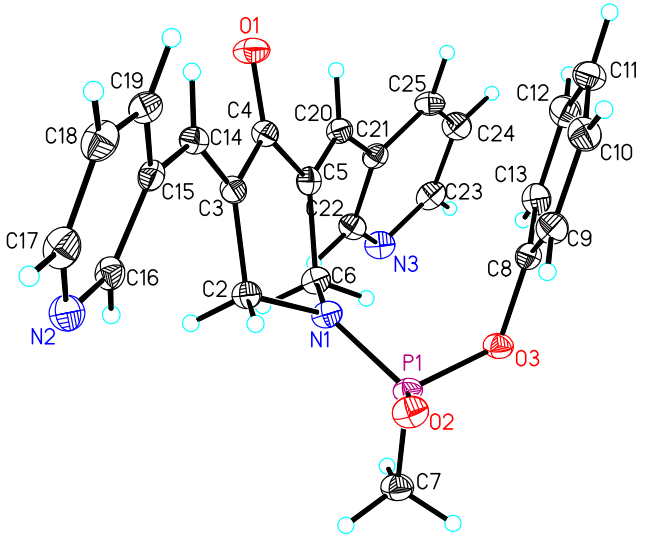


Fig. 2. Molecular structure of **3c** drawn at 30% probability of thermal ellipsoids.

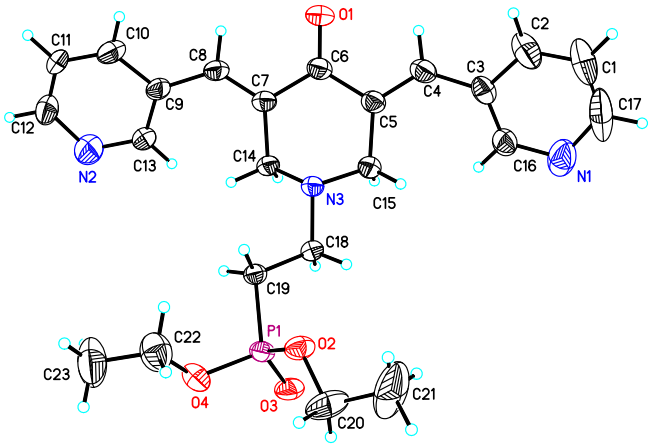


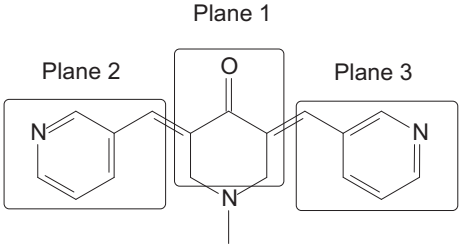
Fig. 3. Molecular structure of **8b** drawn at 50% probability of thermal ellipsoids.

planes, and deviation of the nitrogen atom from the central fragment are presented in Table 1. These data show that central fragments in all molecules have envelope conformation with significant deviation of the N atom from the plane of the central fragment. Values of dihedral angles show that conjugated pharmacophore fragment (see, for instance [18]) in molecule **2a** is more close to planarity than in molecules **3c** and **8b**. Deviation from planarity in two latter molecules might be caused by larger size of substituent at the piperidone N atom as well as by unusual conformation of the substituent in the molecule **3c**.

Previously, it was pointed out [19] that the higher cytotoxicity of a particular bis(aryliden)piperid-4-one might be connected with the planarity of the piperidone nitrogen atom. Presented data on molecular geometrical characteristics for compounds **2a**, **3c**, and **8b** (Table 2) support this supposition as cytotoxic properties of amidophosphonate **3c** are markedly higher comparing with those for N-methyl- and N-phosphorylethyl-substituted analogues **2a** and **8b**, respectively (see Table 3 below). On the other hand, is seems that planarity of conjugated pharmacophore fragment is less significant for cytotoxicity of a compound. Here we are considering just small number of factors responsible for sample bioactivity and more data should be acquired before proper structure–properties relationship will be established.

2.3. Cytotoxic properties

The cytotoxic activity of the compounds synthesized was tested against human cancer cell lines, namely Caov3 (ovarian carcinoma), A549 (lung carcinoma), KB 3-1 (human oral epidermoid carcinoma) and drug resistant subclone of the latter one, i.e. KB 8-5, with MDR1 hyperexpression. The results are summarized in Table 3 showing the corresponding IC_{50} values (IC_{50} is the concentration of compound required to inhibit the growth of the cells by 50%).



Scheme 5. Planar fragments in the molecules of 3,5-bis(pyridinylmethylene)-piperid-4-ones.

Table 1
Dihedral angles between planar fragments in molecules **2a**,**3c** and **8b** shown in Scheme 4, mean atomic deviations from these planes, and deviation of the nitrogen atom from mean plane of the piperidone fragment.

Compound	Dihedral angles between planes, deg.			Mean atomic deviations from planes, Å			Deviation of N from Plane 1, Å
	1/2	1/3	2/3	Plane 1	Plane 2	Plane 3	
2a	14.13(6)	12.67(6)	2.71(6)	0.045	0.008	0.011	0.719
3c	30.32(13)	27.60(16)	14.26(20)	0.015	0.005	0.010	0.701
8b	27.56(8)	36.64(7)	18.52(10)	0.023	0.009	0.010	0.639

Anticancer agent Melphalan (Sarcolysin, alkylation agent) was used as a positive control similar to assays of cytotoxic properties of other 3,5-bis(arylidene)-4-piperidone derivatives described in literature (see, *e.g.*, Ref.[1–5,16–20]). As the second positive control, we use Doxorubicin (anthracycline antibiotic having different mechanism of action). For comparison, the activities of parent NH-piperidones **1a**,**b** and the related N-Me substituted analogues **2a**,**b** were estimated in the same assay in order to estimate the influence of N-alkylation on cytotoxic properties.

In general, cytotoxicity of all 3,5-bis(pyridinylmethylene) piperid-4-ones tested in this assay towards ovarian and lung carcinoma cell lines, CaoV3 and A549, was significantly higher comparing with that of Melphalan. As one can see, compounds bearing 4-pyridinyl moieties on the whole are more active than their analogues having 3-pyridinyl groups. This fact is in agreement with higher electron-withdrawing properties of 4-pyridinyl compared with that of 3-pyridinyl (σ_P = 0.44 and 0.25, respectively).

Introduction of methyl group at the nitrogen atom of 3,5-bis (pyridinylmethylene)piperid-4-ones resulted in significant decrease of antitumor properties towards ovarian and carcinoma cell lines for compound **2a** with 3-pyridine ring while its 4-pyridine containing analogue **2b** possesses even higher activity comparing with that of NH-piperidone **1b**.

As for introduction of a phosphorus moiety, in general its influence was more pronounced for the first series of compounds bearing 3-pyridinyl moieties. Among the derivatives bearing the direct N–P bond, the activities of amidophosphate **3a** and aminophosphonates **3b**, **3c** towards CaoV3 and A549 cell lines were comparable with those for the parent NH-piperidone **1a** while compounds **3a**, **3b** possess higher activity towards KB lines including drug resistant KB 8-5. In a series of ω-aminophosphonates, compound **8c** with longer alkylene chain demonstrated excellent cytotoxic properties comparable with that for **3a**, **3b** while β-aminophosphonate **8b** was less active comparing with the parent NH-piperidone **1a**. At the same time, compounds having more electron-withdrawing 4-pyridinyl rings possess in general the same order of activity independently on the nature of the substituent at the piperidone nitrogen atom.

It is known that expression of transmembrane transporting P-glycoprotein (permeability glycoprotein abbreviated as Pgp170) being responsible for elimination of hydrophobic compounds including the cytostatics, from tumor cells, is a plausible reason of the classical multi-drug resistances in the treatment of cancers [23]. Usually, to overcome such resistance the inhibitors of Pgp170 should be added to cytostatic compounds but till now the suitable and multi-purpose inhibitors were not found out. Therefore, resistant tumors are still difficult to treat. The cell lines KB 3-1 and KB 8-5 differ from each other by expression of the above Pgp170. Mostly, the compounds under investigation are less active to the drug resistant line KB 8-5 comparing KB 3-1 similar to Doxorubicin. However, compounds **3a**, **3c**, and **4c** have shown the similar toxicity towards these cell lines. Such data allowed us to suggest that piperidones **3a**, **3c**, and **4c** are not the substrates for Pgp170 and hence can be of interest for treatment of resistant tumors. However, this problem requires the additional detailed studies.

Table 2
Configuration of the nitrogen atom and positions of the substituents in the compounds **2a**, **3c** and **8b**.

Compound	Bond angles at nitrogen atom, deg.		Configuration of nitrogen atom	Position of phosphorus group
2a	C(8)–N(2)–C(9)	109.73(9)	Trigonal-pyramidal	Equatorial
	C(9)–N(2)–C(18)	111.05(9)		
	C(8)–N(2)–C(18)	110.57(10)		
	Sum	331.35(28)		
3c	C(2)–N(1)–C(6)	113.35(13)	Planar	Pseudo-axial
	C(2)–N(1)–P(1)	121.83(11)		
	C(6)–N(1)–P(1)	123.24(11)		
	Sum	358.42(35)		
8b	C(14)–N(3)–C(15)	110.21(19)	Trigonal-pyramidal	Equatorial
	C(14)–N(3)–C(18)	111.87(18)		
	C(15)–N(3)–C(15)	109.18(18)		
	Sum	331.26(55)		

3. Conclusion

This study has demonstrated that introduction of pyridine rings into the molecule of 3,5-bis((hetero)arilidene)piperid-4-ones resulted in compounds possessing excellent antitumor properties towards both ordinary carcinoma cell lines and drug resistant subclone KB 8-5. The antitumor properties significantly exceeded those for the analogues of these compounds bearing thienylidene rings and those having *E*,*E*-3,5-bis(4-pyridinylmethylene)piperid-4-one framework displayed higher growth inhibitory properties comparing with those with 3-pyridine rings. Introduction of the phosphorus moiety substantially increased the antitumor properties in the case of *E*,*E*-3,5-bis(3-pyridinylmethylene)piperid-4-one derivatives but it is less pronounced for more active analogues bearing 4-pyridinyl rings. That means the high potential 3,5-bis (heteroarylidene)-4-piperidones especially phosphorylated ones as potential drug-candidates for further development to improve activity and bioavailability of 3,5-bis(arylidene)piperid-4-ones.

4. Experimental protocols

4.1. General remarks

NMR spectra were recorded with a Bruker AMX-400 spectrometer (^{1}H, 400.13; ^{19}F, 376.3; ^{31}P, 161.97 and ^{13}C, 100.61 MHz) using residual proton signals (^{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. The numeration for carbon atoms is shown in Scheme 2.

Column chromatography was carried out using Merck silica gel 60 (230–400 mesh ASTM). Analytical TLCs were performed with Merck silica gel 60 F_{254} plates. Visualization was accomplished by

Table 3
Cytotoxicity of phosphorylated 3,5-bis(pyridinylmethylene)-4-piperidones **3a**–**c**, **4a**,**c**, **8b**,**c** and the related NH- and N–Me piperidones **1a**,**b** and **2a**,**b** against human Caov3, A549, KB 3-1, and KB 8-5 cell lines.

Compound	Het	R	CaoV3	A549	KB 3-1	KB 8-5
1a	3-Py	H	9.2 ± 0.1	11.1 ± 0.6	24.4 ± 0.7	30.0 ± 2.3
2a	3-Py	Me	>20	>20	20.2 ± 0.4	40.4 ± 1.7
3a	3-Py	$P(O)(EtO)_2$	8.1 ± 0.3	10.4 ± 0.2	5.0 ± 0.2	5.3 ± 0.4
3b	3-Py	P(O)(EtO)Me	10.8 ± 0.6	11.3 ± 0.4	20.3 ± 0.6	45.8 ± 5.1
3c	3-Py	P(O)(PhO)Me	9.3 ± 0.3	7.5 ± 0.3	7.8 ± 0.1	7.6 ± 0.2
8b	3-Py	$(CH_2)_2P(O)(OEt)_2$	>40	>40	>40	>40
8c	3-Py	$(CH_2)_4P(O)(OEt)_2$	6.2 ± 0.1	15.4 ± 0.2	6.1 ± 0.2	9.5 ± 0.5
1b	4-Py	H	6.0 ± 0.1	10.3 ± 0.1	5.0 ± 0.2	8.5 ± 0.1
2b	4-Py	Me	5.1 ± 0.3	4.2 ± 0.1	3.0 ± 0.2	7.4 ± 0.1
4a	4-Py	$P(O)(EtO)_2$	4.3 ± 0.2	8.2 ± 0.1	2.1 ± 0.1	5.1 ± 0.4
4c	4-Py	P(O)(PhO)Me	7.0 ± 0.3	8.3 ± 0.2	7.1 ± 0.1	7.1 ± 0.2
Melphalan			50 ± 10	50 ± 10	–	–
Doxorubicin			–	0.8	1.2 ± 0.1	3.3 ± 0.1

UV light. IR spectra were recorded in KBr pellets on a Fourier-spectrometer "Magna-IR750" (Nicolet), resolution 2 cm^{-1}, 128 scans. Melting points were determined with MPA 120 EZ-Melt Automated Melting Point Apparatus and were uncorrected.

The reaction mixtures were stirred magnetically in round-bottom flasks, and the reaction course was monitored by ^{19}F or ^{31}P NMR technique or by thin-layer chromatography as appropriate.

All commercial reagents were used as purchased without further purification, all solvents were reagent grade. Ethyl methylphosphonochloridate [24], phenyl methylphosphonochloridate [25], diethyl (1,4-dioxa-8-azaspiro[4.5]dec-8-yl)methylphosphonate 5 [20], diethyl 2-(4-oxopiperidin-1-yl)ethylphosphonate 6a [20], diethyl 4-(4-oxopiperidin-1-yl)butylphosphonate 6b [20] were obtained by the known procedures. (3*E*,5*E*)-1-Methyl-4-oxo-3,5-bis (3-pyridinylmethylene)piperidinone **2a** and (3*E*,5*E*)-1-methyl-4-oxo-3,5-bis(4-pyridinylmethylene)piperidinone **2b** were obtained via aldol-crotonic condensation of N-methyl-piperid-4-one with appropriate pyridinecarboxaldehyde in the presence of $BF_3 \cdot Et_2O$ followed by neutralization of the corresponding tris(tetrafluoroborate) salts [21] and their physical–chemical constants and spectral data fit well the literature data for the same compounds obtained via the aldol-crotonic condensation performed under basic conditions [22].

4.2. (3E,5E)-4-Oxo-3,5-bis(3-pyridinylmethylene)piperidine (*1a*)

Boron trifluoride etherate (4 mmol) was added to a solution of 4-piperidone monohydrate hydrochloride (1 mmol) and the 3-pyridinecarboxaldehyde (2 mmol) in CH_3CN (2 ml). The mixture was stirred at room temperature for 2 days (until consumption of the starting compounds as monitored by TLC). Then the mixture was dissolved in hot EtOH. This solution was cooled to room temperature resulting in formation of crystalline precipitate. The precipitate was filtered off and air-dried to give (*3E,5E)-4-oxo-3,5-bis(3-pyridinylmethylene)piperidinium tris(tetrafluoroborate)* (***1a**·3HBF*$_4$) as a yellow solid, mp ca. 160 °C (decomp.). Yield 84%. IR (KBr, ν, cm^{-1}): 3584, 3194, 3103, 2872, 1687 (C=O), 1629 (C=C), 1593, 1561, 1467, 1305, 1195, 1084, 1059, 980, 817, 679, 544, 523. ^1H (DMSO-d_6), δ, ppm: 4.59 (s, 4H, cyclic NCH_2), 8.01 (s, 2H, C^7-H, $C^{7'}$-H), 8.12 (dd, 2H, $^3J_{HH} = 5.6$ Hz, $^3J_{HH} = 8.0$ Hz, C^{10}-H, $C^{10'}$-H), 8.61 (d, 2H, $^3J_{HH} = 8.3$ Hz, C^9-H, $C^{9'}$-H), 8.94 (d, 2H, $^3J_{HH} = 5.4$ Hz, C^{11}-H, $C^{11'}$-H), 9.10 (s, 2H, C^{13}-H, $C^{13'}$-H), 9.50 (s, HBF_4). ^{13}C (DMSO-d_6), δ, ppm: 43.93 (C^2, C^6), 127.01 (C^{10}, $C^{10'}$), 131.95 (C^3, C^5), 132.58 (C^8, $C^{8'}$), 133.82 (C^9, $C^{9'}$), 143.55 (C^7, $C^{7'}$), 144.46 and 145.22 (C^{13}, $C^{13'}$ and C^{11}, $C^{11'}$), 181.81 (C^4). ^{19}F (DMSO-d_6), δ, ppm: −147.98,-148.04.Anal. calcd. for $C_{17}H_{15}N_3O \cdot 3HBF_4$ (%): C 37.76, H 3.36, N 7.77. Found (%): C 37.72, H 2.98, N 7.42.

For isolation of **1a** as a free base, the corresponding tris(tetrafluoroborate) salt (1.00 g, 0.00185 mol) was taken up into biphasic system CH_2Cl_2 (15 ml)/aqueous Na_2CO_3 (0.72 g, 0.0068 mol, 10 ml H_2O). Reaction mixture was stirred for 1.5 h and then solid NaCl was added. After stirring for 15 min, the layers were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, filtered off and the filtrate was evaporated at reduced pressure to give 0.51 g (100%) of **1a** as a yellow powder, mp 166–175 °C (decomp.). ^1H ($CDCl_3$), δ, ppm: 1.80 (s, 1H, NH), 4.14 (s, 4H, cyclic NCH_2), 7.34 (dd, 2H, $^3J_{HH} = 5.0$ Hz, $^3J_{HH} = 8.0$ Hz, C^{10}-H, $C^{10'}$-H), 7.66 (d, 2H, $^3J_{HH} = 8.0$ Hz, C^9-H, $C^{9'}$-H), 7.74 (s, 2H, C^7-H, $C^{7'}$-H), 8.57 (d, 2H, $^3J_{HH} = 5.0$ Hz, C^{11}-H, $C^{11'}$-H), 8.63 (s, 2H, C^{13}-H, $C^{13'}$-H). Anal. calcd. for $C_{17}H_{15}N_3O$ (%): C 73.63, H 5.45, N 15.15. Found (%): C 73.38, H 5.44, N 14.95.

4.3. (3E,5E)-4-Oxo-3,5-bis(4-pyridinylmethylene)piperidine (*1b*)

The compound was obtained according to the procedure similar to that for the synthesis of its 3-pyridinyl analogue **1a**·excluding the reaction time for condensation being 7 days (r.t.) to afford (*3E,5E)-4-oxo-3,5-bis(4-pyridinylmethylene)piperidinium tris(tetrafluoroborate)* (***1b**·3HBF*$_4$) as an yellow solid in 21% yield. Mp 249 °C (decomp.). IR (KBr, ν, cm^{-1}): 3287, 3187, 3111, 1700 (C=O), 1634 (C=C), 1615, 1502, 1322, 1305, 1239, 1190, 1084, 1064, 925, 760, 746, 524. ^1H (DMSO-d_6), δ, ppm: 4.57 (s, 4H, cyclic NCH_2), 8.02 (s, 2H, C^7-H, $C^{7'}$-H), 8.13 (d, 4H, $^3J_{HH} = 6.0$ Hz, C^9-H, $C^{9'}$-H, C^{13}-H, $C^{13'}$-H), 9.01 (d, 4H, $^3J_{HH} = 6.2$ Hz, C^{10}-H, $C^{10'}$-H, C^{12}-H, $C^{12'}$-H), 12.43 (s, HBF_4). ^{13}C (DMSO-d_6), δ, ppm: 43.97 (C^2, C^6), 127.27 (C^9, C^{13}, $C^{9'}$, $C^{13'}$), 134.22 (C^3, C^5), 134.79 (C^7, $C^{7'}$), 143.05 (C^{10}, C^{12}, $C^{10'}$, $C^{12'}$), 149.95 (C^8, $C^{8'}$), 181.68 (C^4). Anal. calcd. for $C_{17}H_{15}N_3O \cdot 3HBF_4$ (%): C 37.76, H 3.36, N 7.77. Found (%): C 37.76, H 3.83, N 7.79.

Further neutralization provided **1b** as a free base in 94% yield based on the intermediate tris(tetrafluoroborate) salt. ^1H ($CDCl_3$), δ, ppm: 1.83 (s, 1H, NH), 4.12 (s, 4H, cyclic NCH_2), 7.20 (d, 4H, $^3J_{HH} = 5.0$ Hz, C^9-H, $C^{9'}$-H, C^{13}-H, $C^{13'}$-H), 7.65 (s, 2H, C^7-H, $C^{7'}$-H), 8.65 (d, 4H, $^3J_{HH} = 5.0$ Hz, C^{10}-H, $C^{10'}$-H, C^{12}-H, $C^{12'}$-H). ^{13}C ($CDCl_3$), δ, ppm: 47.61 (C^2, C^6), 123.82 (C^9, C^{13}, $C^{9'}$, $C^{13'}$), 132.98 (C^3, C^5), 137.72 (C^7, $C^{7'}$), 141.97 (C^{10}, C^{12}, $C^{10'}$, $C^{12'}$), 149.97 (C^8, $C^{8'}$), 186.82 (C^4). Analytically pure sample was obtained by column chromatography using CH_2Cl_2/EtOH = 100:15 as an eluent. Anal. calcd. for $C_{17}H_{15}N_3O$ (%): C 73.63, H 5.45, N 15.15. Found (%): C 73.66, H 5.28, N 15.19.

4.4. Phosphorylation of 4-oxo-3,5-bis(pyridinylmethylene)-1-piperidinones *1a,b* (general procedure)

To a mixture of the corresponding NH-3,5-bis(pyridinylmethylene)piperidone **1a**,**b** (1.8 mmol) and triethylamine (2.3 mmol) in dry tetrahydrofuran (9 ml) a solution of corresponding chlorophosphonate or chlorophosphate (2.2 mmol) in dry tetrahydrofuran (4 ml) was added. The reaction mixture was stirred at

room temperature over 24 h. The reaction was monitored by TLC and the ^{31}P NMR technique. After completion of the reaction, the mixture was evaporated at reduced pressure, dissolved in CH_2Cl_2 and washed with water. The organic layer was separated, evaporated at reduced pressure and purified by column chromatography (SiO_2, THF or mixture of CH_2Cl_2/EtOH = 100:5 as an eluent). The appropriate fractions were evaporated under reduced pressure to give the solid product. If desired, the product can be additionally purified by precipitation with pentane from solution in CH_2Cl_2.

4.4.1. Diethyl (3E,5E)-4-oxo-3,5-bis(3-pyridinylmethylene)-1-piperidinylphosphonate **3a**

Yellow solid, mp 122–124 °C. Yield 52%. IR (KBr, ν, cm^{-1}): 2984, 1669 (C=O), 1614(C=C), 1587, 1565, 1480, 1415, 1269, 1256, 1241, 1212, 1154, 1047, 1023, 970, 960, 807, 769, 702, 628, 531. ^1H ($CDCl_3$), δ, ppm: 1.15 (t, 6H, $^3J_{HH}$ = 7.0 Hz, $P(OCH_2\underline{CH_3})_2$), 3.81–4.03 (m, 4H, $P(O\underline{CH_2}CH_3)_2$), 4.43 (d, 4H, $^3J_{HP}$ = 8.2 Hz, cyclic NCH_2), 7.36 (dd, 2H, $^3J_{HH}$ = 4.9 Hz, $^3J_{HH}$ = 7.9 Hz, C^{10}-H, $C^{10'}$-H), 7.69 (d, 2H, $^3J_{HH}$ = 7.9 Hz, C^9-H, $C^{9'}$-H), 7.76 (s, 2H, C^7-H, $C^{7'}$-H), 8.58 (d, 2H, $^3J_{HH}$ = 4.7 Hz, C^{11}-H, $C^{11'}$-H), 8.64 (s, 2H, C^{13}-H, $C^{13'}$-H). ^{13}C ($CDCl_3$), δ, ppm: 15.85 (d, $^3J_{PC}$ = 6.9 Hz, $P(OCH_2\underline{CH_3})_2$), 46.02 (d, $^2J_{PC}$ = 3.6 Hz, C^2, C^6), 62.72 (d, $^2J_{PC}$ = 5.5 Hz, $P(O\underline{CH_2}CH_3)_2$), 123.48 ($C^{10}$, $C^{10'}$), 130.36 (C^8, $C^{8'}$), 133.30 (C^7, $C^{7'}$), 133.95 (d, $^3J_{PC}$ = 4.9 Hz, C^3, C^5), 136.83 (C^9, $C^{9'}$), 149.89 and 151.00 (C^{13}, $C^{13'}$ and C^{11}, $C^{11'}$), 185.75 (C^4). ^{31}P ($CDCl_3$), δ, ppm: 7.21. Anal. calcd. for $C_{21}H_{24}N_3O_4P$: C, 61.01; H, 5.85; N, 10.16%. Found: C, 60.68; H, 6.13; N, 9.98%.

4.4.2. Ethyl methyl[(3E,5E)-4-oxo-3,5-bis(3-pyridinylmethylene)-1-piperidinyl]-phosphinate **3b**

Yellow solid, mp 131–139 °C (decomp.). Yield 60%. IR (KBr, ν, cm^{-1}): 3085, 2925, 1726, 1665 (C=O), 1612 (C=C), 1579, 1560, 1477, 1420, 1309, 1269, 1240, 1197, 1186, 1155, 1072, 1034, 987, 959, 939, 902, 810, 755, 709, 695, 632б 545. ^1H ($CDCl_3$), δ, ppm: 1.07 (t, 3H, $^3J_{HH}$ = 7.1 Hz, $POCH_2\underline{CH_3}$), 1.29 (d, 3H, $^2J_{HP}$ = 18.4 Hz, PCH_3), 3.67–3.73 (m, 1H, OCH_2), 3.86–3.92 (m, 1H, OCH_2), 4.40 (d, 4H, $^3J_{HP}$ = 8.0 Hz, cyclic NCH_2), 7.32 (dd, 2H, $^3J_{HH}$ = 4.9 Hz, $^3J_{HH}$ = 8.0 Hz, C^{10}-H, $C^{10'}$-H), 7.66 (d, 2H, $^3J_{HH}$ = 8.0 Hz, C^9-H, $C^{9'}$-H), 7.73 (s, 2H, C^7-H, $C^{7'}$-H), 8.54 (d, 2H, $^3J_{HH}$ = 4.6 Hz, C^{11}-H, $C^{11'}$-H), 8.60 (s, 2H, C^{13}-H, $C^{13'}$-H). ^{13}C ($CDCl_3$), δ, ppm: 11.91 (d, $^1J_{PC}$ = 133 Hz, PCH_3), 15.86 (d, $^3J_{PC}$ = 6.6 Hz, $POCH_2\underline{CH_3}$), 45.29 (d, $^2J_{PC}$ = 4.4 Hz, C^2, C^6), 59.77 (d, $^2J_{PC}$ = 6.6 Hz, $PO\underline{CH_2}CH_3$), 123.45 (C^{10}, $C^{10'}$), 130.18 (C^8, $C^{8'}$), 133.37 (C^7, $C^{7'}$), 133.83 (d, $^3J_{PC}$ = 3.7 Hz, C^3, C^5), 136.78 (C^9, $C^{9'}$), 149.89 and 150.91 (C^{13}, $C^{13'}$ and C^{11}, $C^{11'}$), 185.65 (C^4). ^{31}P ($CDCl_3$), δ, ppm: 31.52. Anal. calcd. for $C_{20}H_{22}N_3O_3P\cdot 0.8H_2O$ (%): C 60.39, H 5.98, N 10.56, P 7.79. Found (%): C 60.69, H 5.48, N 10.07, P 7.72.

4.4.3. Phenyl methyl[(3E,5E)-4-oxo-3,5-bis(3-pyridinylmethylene)-1-piperidinyl]-phosphinate **3c**

The compound was purified by column chromatography using gradient elution from 100% CH_2Cl_2 to CH_2Cl_2/EtOH = 20/1 to give **3c** as a strong solvate with ethanol which was observed in the ^1H NMR spectra. Yellow solid, mp. >99 °C (decomp.). Yield 51%. IR (KBr, ν, cm^{-1}): 3429, 3054, 2916, 1733, 1670 (C=O), 1613 (C=C), 1585, 1564, 1495, 1482, 1415, 1329, 1310, 1274, 1240, 1200, 1187, 1167, 1159, 1070, 1026, 987, 917, 907, 893, 814, 774, 764, 715, 693, 566, 550, 514, 475. ^1H ($CDCl_3$), δ, ppm: 1.50 (d, 3H, $^2J_{HP}$ = 16.6 Hz, PCH_3), 4.47 (d, 4H, $^3J_{HP}$ = 8.3 Hz, cyclic NCH_2), 6.97 (d, 2H, $^3J_{HH}$ = 8.4 Hz, *o*-H in C_6H_5O), 7.12 (t, 1H, $^3J_{HH}$ = 7.3 Hz, *p*-H in C_6H_5O), 7.25 (d, 2H, $^3J_{HH}$ = 7.8 Hz, *m*-H in C_6H_5O), 7.38 (dd, 2H, $^3J_{HH}$ = 5.0 Hz, $^3J_{HH}$ = 7.8 Hz, C^{10}-H, $C^{10'}$-H), 7.68 (d, 2H, $^3J_{HH}$ = 7.8 Hz, C^9-H, $C^{9'}$-H), 7.70 (s, 2H, C^7-H, $C^{7'}$-H), 8.61 (dd, 2H, $^3J_{HH}$ = 5.3 Hz, C^{11}-H, $C^{11'}$-H), 8.62 (s, 2H, C^{13}-H, $C^{13'}$-H). ^{13}C ($CDCl_3$), δ, ppm: 12.33 (d, $^1J_{PC}$ = 136 Hz, PCH_3), 45.20 (d, $^2J_{PC}$ = 4.4 Hz, C^2, C^6), 119.80 (d, $^3J_{PC}$ = 4.9 Hz, *o*-C in C_6H_5O), 123.52 (C^{10}, $C^{10'}$), 124.75 (*p*-C in C_6H_5O), 129.66 (*m*-C in C_6H_5O), 130.23 (C^8, $C^{8'}$), 133.43 (C^3, C^5), 133.45 (d, $^4J_{PC}$ = 4.9 Hz, C^7, $C^{7'}$), 136.89 (C^9, $C^{9'}$), 149.95 (C^{13}, $C^{13'}$), 150.88 (C^{11}, $C^{11'}$), 185.16 (C^4). ^{31}P ($CDCl_3$), δ, ppm: 29.80. Anal. calcd. for $C_{24}H_{22}N_3O_3P\cdot 0.75EtOH$ (%): C 65.73, H 5.73, N 9.02. Found (%): C 65.56, H 5.64, N 8.83.

4.4.4. Diethyl (3E,5E)-4-oxo-3,5-bis(4-pyridinylmethylene)-1-piperidinylphosphonate **4a**

Yellow solid, mp 97.5–99.5 °C. Yield 62%. IR (KBr, ν, cm^{-1}): 2982, 1672 (C=O), 1616 (C=C), 1593, 1585, 1544, 1414, 1391, 1365, 1325, 1264, 1253, 1233, 1186, 1150, 1101, 1050, 1021, 965, 940, 858, 821, 754, 690, 626, 537. ^1H ($CDCl_3$), δ, ppm: 1.17 (t, 6H, $^3J_{HH}$ = 7.0 Hz, $P(OCH_2\underline{CH_3})_2$), 3.84–4.04 (m, 4H, $P(O\underline{CH_2}CH_3)_2$), 4.41 (d, 4H, $^3J_{PH}$ = 8.4 Hz, cyclic NCH_2), 7.23 (d, 4H, $^3J_{HH}$ = 4.4 Hz, C^9-H, $C^{9'}$-H, C^{13}-H, $C^{13'}$-H), 7.68 (s, 2H, C^7-H, $C^{7'}$-H), 8.68 (d, 4H, $^3J_{HH}$ = 4.6 Hz, C^{10}-H, $C^{10'}$-H, C^{12}-H, $C^{12'}$-H). ^{13}C ($CDCl_3$), δ, ppm: 15.88 (d, $^3J_{PC}$ = 7.0 Hz, $P(OCH_2\underline{CH_3})_2$), 45.92 (d, $^2J_{PC}$ = 3.7 Hz, C^2, C^6), 62.83 (d, $^2J_{PC}$ = 5.9 Hz, $P(O\underline{CH_2}CH_3)_2$), 123.71 ($C^9$, C^{13}, $C^{9'}$, $C^{13'}$), 134.11 (C^7, $C^{7'}$), 135.48 (d, $^3J_{PC}$ = 6.1 Hz, C^3, C^5), 141.66 (C^8, $C^{8'}$), 150.26 (C^{10}, C^{12}, $C^{10'}$, $C^{12'}$), 185.86 (C^4). ^{31}P ($CDCl_3$), δ, ppm: 7.20. Anal. calcd. for $C_{21}H_{24}N_3O_4P$ (%): C 61.01, H 5.85, N 10.16. Found (%): C 60.88, H 5.67, N 9.94.

4.4.5. Ethyl methyl[(3E,5E)-4-oxo-3,5-bis(4-pyridinylmethylene)-1-piperidinyl] phosphinate **4b**

Yellow solid, mp 60–62 °C. Yield 12%, purity according to the ^1H NMR data ~89%. IR (KBr, ν, cm^{-1}): 3030, 2982, 1661 (C=O), 1644, 1594, 1547, 1528, 1506, 1443, 1415, 1390, 1362, 1327, 1308, 1280, 1267, 1223, 1183, 1164, 1102, 1040, 995, 958, 903, 788, 754, 533, 494. ^1H ($CDCl_3$), δ, ppm: 1.20 (t, 3H, $^3J_{HH}$ = 7.0 Hz, $POCH_2\underline{CH_3}$), 1.41 (d, 3H, $^2J_{HP}$ = 16.3 Hz, PCH_3), 3.79–3.85 (m, 1H, OCH_2), 3.97–4.18 (m, 1H, OCH_2), 4.48 (d, 4H, $^3J_{HP}$ = 8.0 Hz, cyclic NCH_2), 7.31 (d, 4H, $^3J_{HH}$ = 5.8 Hz, C^9-H, $C^{9'}$-H, C^{13}-H, $C^{13'}$-H), 7.76 (s, 2H, C^7-H, $C^{7'}$-H), 8.74 (d, 4H, $^3J_{HH}$ = 5.7 Hz, C^{10}-H, $C^{10'}$-H, C^{12}-H, $C^{12'}$-H). ^{13}C ($CDCl_3$), δ, ppm: 11.89 (d, $^1J_{PC}$ = 132.8 Hz, PCH_3), 15.91 (d, $^3J_{PC}$ = 6.6 Hz, $POCH_2\underline{CH_3}$), 45.16 (d, $^2J_{PC}$ = 3.8 Hz, C^2, C^6), 59.89 (d, $^2J_{PC}$ = 6.6 Hz, $PO\underline{CH_2}CH_3$), 123.65 (C^9, C^{13}, $C^{9'}$, $C^{13'}$), 134.21 (C^7, $C^{7'}$), 135.35 (d, $^3J_{PC}$ = 3.3 Hz, C^3, C^5), 141.44 (C^8, $C^{8'}$), 150.22 (C^{10}, C^{12}, $C^{10'}$, $C^{12'}$), 185.77 (C^4). ^{31}P ($CDCl_3$), δ, ppm: 31.68.

4.4.6. Phenyl methyl[(3E,5E)-4-oxo-3,5-bis(4-pyridinylmethylene)-1-piperidinyl]phosphinate **4c**

Yellow solid, mp 52–57 °C. Yield 43%. IR (KBr, ν, cm^{-1}): 3420, 3038, 2921, 2853, 1660 (C=O), 1643 (C=C), 1593, 1554, 1527, 1490, 1414, 1309, 1264, 1234, 1205, 1187, 1164, 1110, 1064, 1025, 995, 984, 917, 898, 813, 775, 766, 692, 531, 504, 474. ^1H ($CDCl_3$), δ, ppm: 1.55 (d, 3H, $^2J_{HP}$ = 16.5 Hz, PCH_3), 4.49 (d, 4H, $^3J_{HP}$ = 8.4 Hz, cyclic NCH_2), 7.01 (d, 2H, $^3J_{HH}$ = 7.4 Hz, *o*-H in C_6H_5O), 7.17 (t, 1H, $^3J_{HH}$ = 7.3 Hz, *p*-H in C_6H_5O), 7.25–7.28 (m, 4H, *m*-H in C_6H_5O and C^9-H, $C^{9'}$-H, C^{13}-H, $C^{13'}$-H), 7.65 (s, 2H, C^7-H, $C^{7'}$-H), 8.73 (d, 4H, $^3J_{HH}$ = 5.5 Hz, C^{10}-H, $C^{10'}$-H, C^{12}-H, $C^{12'}$-H). ^{13}C ($CDCl_3$), δ, ppm: 12.27 (d, $^1J_{PC}$ = 135.6 Hz, PCH_3), 45.00 (d, $^2J_{PC}$ = 3.8 Hz, C^2, C^6), 119.73 (d, $^3J_{PC}$ = 4.9 Hz, *o*-C in C_6H_5O), 123.63 (C^9, C^{13}, $C^{9'}$, $C^{13'}$), 124.77 (*p*-C in C_6H_5O), 129.64 (*m*-C in C_6H_5O), 134.20 (C^7, $C^{7'}$), 134.85 (d, $^3J_{PC}$ = 3.3 Hz, C^3, C^5), 141.44 (C^8, $C^{8'}$), 150.13 (C^{10}, C^{12}, $C^{10'}$, $C^{12'}$), 185.10 (C^4). ^{31}P ($CDCl_3$), δ, ppm: 29.79. Anal. calcd. for $C_{24}H_{22}N_3O_3P\cdot H_2O$ (%): C 64.14, H 5.39, N 9.35. Found (%): C 63.96, H 5.03, N 9.11.

4.5. (3E,5E)-1-[(Diethoxyphosphoryl)methyl]-4-oxo-3,5-bis(3-pyridinylmethylene)-piperidinium tetrafluoroborate **7a**

Boron trifluoride etherate (4 ml) was added to a mixture of diethyl (1,4-dioxa-8-azaspiro[4.5]dec-8-yl)methyl-phosphonate **6a** (0.28 g, 1 mmol) and 3-pyridinaldehyde (0.20 g, 2 mmol) cooled on ice-bath. The resulting mixture was stirred at room temperature for 7 days. Addition of Et_2O (15 ml) resulted in the formation of the oily residue. The ether layer was discarded and the crude product was recrystallized from MeOH followed by trituration in Et_2O to afford

7a (0.2 g, 34%) as a yellow powder. Mp. >156 °C (decomp.). IR (KBr, ν, cm^{-1}): 3434, 3129, 2983, 2928, 2599, 1676, 1632, 1598, 1550, 1465, 1265, 1235, 1205, 1124, 1084, 1063, 992, 814, 792, 766, 681, 522. ^1H (DMSO-d_6), δ, ppm: 1.11 (t, $^3J_{HH}$ = 7.0 Hz, 6H, P(OCH$_2$CH$_3$)$_2$), 3.16 (d, $^2J_{HP}$ = 11.5 Hz, 2H, NCH$_2$P), 3.86–3.99 (m, 4H, P(OCH$_2$CH$_3$)$_2$), 4.18 (s, 4H, cyclic NCH$_2$), 5.07 (s, HBF_4), 7.78 (s, 2H, C^7-H, $C^{7'}$-H), 7.93 (dd, 2H, $^3J_{HH}$ = 5.6 Hz, $^3J_{HH}$ = 8.0 Hz, C^{10}-H, $C^{10'}$-H), 8.37 (d, 2H, $^3J_{HH}$ = 7.5 Hz, C^9-H, $C^{9'}$-H), 8.83 (d, 2H, $^3J_{HH}$ = 5.2 Hz, C^{11}-H, $C^{11'}$-H), 8.97 (s, 2H, C^{13}-H, $C^{13'}$-H). ^{13}C (DMSO-d_6), δ, ppm: 16.49 (d, $^3J_{PC}$ = 5.5 Hz, P(OCH$_2$CH$_3$)$_2$), 51.06 (d, $^1J_{PC}$ = 149 Hz, NCH$_2$P), 54.28 (d, $^2J_{PC}$ = 7.5 Hz, cyclic NCH$_2$), 61.33 (d, $^2J_{PC}$ = 5.8 Hz, P(OCH$_2$CH$_3$)$_2$), 126.16 (C^{10}, $C^{10'}$), 132.17 (C^3, C^5), 132.89 (C^8, $C^{8'}$), 133.73 (C^9, $C^{9'}$), 143.07 (C^7, $C^{7'}$), 145.20 and 146.37 (C^{13}, $C^{13'}$ and C^{11}, $C^{11'}$), 183.38 (C^4). ^{19}F (DMSO-d_6), δ, ppm: −147.98, −148.03. ^{31}P (DMSO-d_6), δ, ppm: 22.82. ^{19}F (DMSO-d_6), δ, ppm: −147.98, −148.03. Anal. calcd. for $C_{22}H_{26}N_3O_4P \cdot 2HBF_4 \cdot H_2O$ (%): C 42.54, H 4.87, N 6.77. Found (%): C 42.46, H 4.68, N 6.74.

4.5.1. (3E,5E)-1-[4-(Diethoxyphosphoryl)butyl]-4-oxo-3,5-bis(3-pyridinylmethylene)piperidinium tris(tetrafluoroborate) **7c**

Boron trifluoride etherate (10 ml) was added to a cooled (ice-bath) mixture of diethyl 3-(4-oxopiperidin-1-yl)butylphosphonate **6c** (0.88 g, 3 mmol) and 3-pyridinecarboxaldehyde (0.64 g, 6 mmol). Formation of oily precipitate was immediately observed. The mixture was stirred at room temperature for 18 h. After addition of Et_2O (15 ml), the upper ether layer was decanted from oily residue, which was recrystallized from a mixture of CH_2Cl_2/MeOH = 10:1 to give 1.27 g (56%) of **7c** as a yellow powder, mp > 160 °C (decomp.). IR (KBr, ν, cm^{-1}): 3429, 3044, 2979, 2933, 2866, 2804, 1669 (C=O), 1649 (C=C), 1619, 1583, 1563, 1479, 1415, 1391, 1276, 1267, 1235, 1203, 1166, 1097, 1052, 1023, 987, 961, 942, 809, 789, 707, 546. ^1H (DMSO-d_6), δ, ppm: 1.20 (t, 6H, $^3J_{HH}$ = 7.0 Hz, P(OCH$_2$CH$_3$)$_2$), 1.45–1.56 (m, 2H, CH$_2$CH$_2$CH$_2$P), 1.69–1.82 (m, 4H, CH$_2$CH$_2$CH$_2$P), 3.33 (t, 2H, $^3J_{HH}$ = 6.9 Hz, NCH$_2$CH$_2$CH$_2$CH$_2$P), 3.94–4.03 (m, 4H, P(OCH$_2$CH$_3$)$_2$), 4.72 (s, 4H, cyclic N(CH$_2$)$_2$), 7.97 (m 4H), 8.43 (s, 2H), 8.89 (s, 2H), 9.03 (s, 2H). ^{13}C (DMSO-d_6), δ, ppm: 16.48 (d, $^3J_{PC}$ = 5.4 Hz, P(OCH$_2$CH$_3$)$_2$), 19.41 (d, $^2J_{PC}$ = 4.3 Hz, CH$_2$CH$_2$CH$_2$CH$_2$P), 24.00 (d, $^1J_{PC}$ = 138 Hz, CH$_2$CH$_2$CH$_2$CH$_2$P), 24.03 (d, $^3J_{PC}$ = 16.2 Hz, CH$_2$CH$_2$CH$_2$CH$_2$P), 51.65 (C^2, C^6), 55.63 (CH$_2$CH$_2$CH$_2$CH$_2$P), 61.20 (d, $^2J_{PC}$ = 6.5 Hz, P(OCH$_2$CH$_3$)$_2$), 127.08 (C^{10}, $C^{10'}$), 131.15 (C^8, $C^{8'}$), 132.37 (C^3, C^5), 134.40 (C^7, $C^{7'}$), 143.53, 144.68, 145.39, 181.21 (C^4). ^{31}P (DMSO-d_6), δ, ppm: 31.10. Anal. calcd. for $C_{25}H_{32}N_3O_4P \cdot 3HBF_4 \cdot H_2O$ (%): C 39.98, H 4.97, N 5.60. Found (%): C 40.27, H 4.68, N 5.64.

Further neutralization of **7c** provided free base **8c** as a viscous semisolid substance. ^1H ($CDCl_3$), δ, ppm: 1.20 (t, 6H, $^3J_{HH}$ = 7.0 Hz, P(OCH$_2$CH$_3$)$_2$), 1.45–1.65 (m, 6H, CH$_2$CH$_2$CH$_2$P), 2.50 (t, 2H, $^3J_{HH}$ = 6.7 Hz, NCH$_2$CH$_2$CH$_2$CH$_2$P), 3.74 (s, 4H, cyclic N(CH$_2$)$_2$), 3.94–4.05 (m, 4H, P(OCH$_2$CH$_3$)$_2$), 7.33 (dd, 2H, $^3J_{HH}$ = 4.9 Hz, $^3J_{HH}$ = 7.9 Hz, C^{10}-H, $C^{10'}$-H), 7.65 (d, 2H, $^3J_{HH}$ = 8.0 Hz, C^9-H, $C^{9'}$-H), 7.69 (s, 2H, C^7-H, $C^{7'}$-H), 8.54 (d, 2H, $^3J_{HH}$ = 3.6 Hz, C^{11}-H, $C^{11'}$-H), 8.60 (s, 2H, C^{13}-H, $C^{13'}$-H). ^{13}C ($CDCl_3$), δ, ppm: 16.22 (d, $^3J_{PC}$ = 6.0 Hz, P(OCH$_2$CH$_3$)$_2$), 20.03 (d, $^2J_{PC}$ = 5.2 Hz, CH$_2$CH$_2$CH$_2$CH$_2$P), 25.14 (d, $^1J_{PC}$ = 140 Hz, CH$_2$CH$_2$CH$_2$CH$_2$P), 27.59 (d, $^3J_{PC}$ = 16.3 Hz, CH$_2$CH$_2$CH$_2$CH$_2$P), 54.47 (C^2, C^6), 56.52 (CH$_2$CH$_2$CH$_2$CH$_2$P), 61.23 (d, $^2J_{PC}$ = 6.4 Hz, P(OCH$_2$CH$_3$)$_2$), 123.32 (C^{10}, $C^{10'}$), 130.77 (C^8, $C^{8'}$), 132.67 (C^7, $C^{7'}$), 134.63 (C^3, C^5), 136.92 (C^9, $C^{9'}$), 149.52 and 150.79 (C^{13}, $C^{13'}$ and C^{11}, $C^{11'}$), 186.19 (C^4). ^{31}P ($CDCl_3$), δ, ppm: 31.72. Anal. calcd. for $C_{25}H_{32}N_3O_4P$ (%): C 63.95, H 6.87, N 8.95. Found (%): C 63.44, H 7.08, N 8.57.

4.5.2. Diethyl 2-[(3E,5E)-4-oxo-3,5-bis(3-pyridinylmethylene) piperidin-1-yl]ethylphosphonate **8b**

A solution of diethyl 2-(4-oxopiperidin-1-yl)ethylphosphonate **6b** (1.65 g, 0.0063 mol), 3-pyridinecarboxaldehyde (1.34 g, 0.0125 mol) in EtOH (5 ml), 8 drops of piperidine and 8 drops glacial acetic acid was refluxed for 4 days. After removing of volatiles under reduced pressure, the oily residue was purified by column chromatography on silica gel. Elution was started with acetone (~150 ml) and continued with THF. The appropriate THF fractions were evaporated under reduced pressure, and the residue was taken up in THF. After addition of Et_2O to this solution, its slow diffusion resulted in precipitation of the product as a yellow crystalline solid, mp 137–144 °C (decomp.). Yield 0.78 g (28%). ^1H ($CDCl_3$), δ, ppm: 1.25 (t, 6H, $^3J_{HH}$ = 7.0 Hz, P(OCH$_2$CH$_3$)$_2$), 1.80–1.98 (m, 2H, CH$_2$P), 2.78–2.91 (m, 2H, CH$_2$CH$_2$P), 3.82 (s, 4H, cyclic N(CH$_2$)$_2$), 3.95–4.10 (m, 4H, P(OCH$_2$CH$_3$)$_2$), 7.37 (dd, 2H, $^3J_{HH}$ = 5.0 Hz, $^3J_{HH}$ = 8.0 Hz, C^{10}-H, $C^{10'}$-H), 7.68 (d, 2H, $^3J_{HH}$ = 8.0 Hz, C^9-H, $C^{9'}$-H), 7.76 (s, 2H, C^7-H, $C^{7'}$-H), 8.59 (d, 2H, $^3J_{HH}$ = 5.0 Hz, C^{11}-H, $C^{11'}$-H), 8.64 (s, 2H, C^{13}-H, $C^{13'}$-H). ^{13}C ($CDCl_3$), δ, ppm: 16.10 (d, $^3J_{PC}$ = 6 Hz, P(OCH$_2$CH$_3$)$_2$), 23.95 (d, $^1J_{PC}$ = 140 Hz, CH$_2$P), 50.42 (s, CH$_2$CH$_2$P), 53.90 (C^2, C^6), 61.37 (d, $^2J_{PC}$ = 6 Hz, P(OCH$_2$CH$_3$)$_2$), 123.21 (C^{10}, $C^{10'}$), 130.49 (C^8, $C^{8'}$), 132.37 (C^7, $C^{7'}$), 134.11 (C^3, C^5), 136.70 (C^9, $C^{9'}$), 149.53 and 150.72 (C^{13}, $C^{13'}$ and C^{11}, $C^{11'}$), 185.66 (C^4). ^{31}P ($CDCl_3$), δ, ppm: 30.66. HRMS: found (M^+) 441.18120, calcd. (M^+) 441.18175.

4.6. X-ray structure determination

The single crystals of **2a** suitable for X-ray experiments were obtained by recrystallization from DCM, by slow diffusion of diethyl ether into the solution in DMSO (**3c**) or in THF (**8b**). Data were collected on a Bruker SMART APEX II CCD diffractometer (l(Mo Kα)-radiation, graphite monochromator, omega and phi scan mode) and corrected for absorption using the SADABS program (version 2.03 [26]). The structures were solved by direct methods and refined by a full-matrix least squares technique on F2 with anisotropic displacement parameters for non-hydrogen atoms. The hydrogen atoms in all compounds were placed in calculated positions and refined within the riding model with fixed isotropic displacement parameters (Uiso(H) ¼ 1.5 Ueq(C) for the CH_3-groups and Uiso(H) ¼ 1.2 Ueq(C) for the other groups). All calculations were carried out using the SHELXTL program [27]. For more details of data collection and structure solution see Table 3. Crystallographic data for **2a**, **3c** and **8b** have been deposited with the CambridgeCrystallographic Data Center. CCDC 776801–776803 contain 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: þ44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk) Table 4.

Table 4
Crystallographic data for **2a**,**3c**,and **8b**.

Compound	**2a**	**3c**	**8b**
Empirical formula	$C_{18}H_{17}N_3O$	$C_{23}H_{28}N_3O_4P$	$C_{24}H_{22}N_3O_3P$
FW	291.35	441.45	431.42
T, K	100(2)	298(2)	136(2)
Crystal system	Triclinic	Triclinic	Triclinic
Space group	P-1	P-1	P-1
a, Å	5.8194(8)	8.529(5)	11.0617(13)
b, Å	9.0874(12)	10.865(6)	11.3621(12)
c, Å	14.3210(19)	12.991(7)	11.5682(14)
α, deg.	102.490(2)	95.126(8)	83.128(3)
β, deg.	96.681(2)	100.524(8)	62.021(3)
γ, deg.	98.004(2)	93.928(8)	62.263(2)
V, Å^3	723.76(17)	1174.5(11)	1127.4(2)
Z	2	2	2
d_c, mg/m^3	1.337	1.248	1.271
F(000)	308	468	452
μ, mm^{-1}	0.085	0.150	0.152
$2\theta_{max}$, deg.	55.98	52	49.98
Reflections collected/unique	7297/3457	13682/4616	3962/3962
$R1$; $wR2$ ($I > 2\sigma(I)$)	0.0390; 0.0982	0.0588; 0.1816	0.0368; 0.0911
$R1$; $wR2$ (all data)	0.0497; 0.1041	0.0770; 0.1980	0.0435; 0.0942
GOF on F^2	1.009	1.374	1.019

4.7. Biological evaluations

Cell lines used for estimation of toxicity of compounds were CaoV3 (human ovarian carcinoma), A549 (human lung carcinoma), KB 3-1 (human oral epidermoid carcinoma cells), and drug resistant subclone of the latter one, i.e. KB 8-5, with MDR1 hyperexpression. 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 of cultivation by the MTT (3-(4,5-dimethyldiazolyl-2)-2,5-diphenyl tetrazolium-bromide) colorimetric assay. The test is based on the ability of mitochondrial dehydrogenase in viable cells to convert MTT reagent (ICN Biomedicals, Germany) into a soluble blue formazan dye. Briefly, the different cell lines were seeded into 96-well plates at a concentration of 1×10^4 cells/100 μl/well. The cells were allowed to attach overnight at 37 °C in a humidified atmosphere containing 5%CO_2. The tested compounds were initially dissolved in dimethylsulfoxide (DMSO) and the working solutions were added to FBS free culture medium. The compounds were added to wells with increasing drug concentrations. After 72 h incubation, 20 μl of MTT reagent (5 mg/ml) were added and cell cultures were incubated for 3 h at 37 °C. After removal of the culture medium formazan crystals were dissolved in dimethylsulfoxide (Sigma–Aldrich) to determine the amount of formazan product. The optic density (OD) was determined by the multi-well plate reader (Uniplan, Picon, Russia) at 590 nm The results were expressed as percent decrease of cell viability as compared to untreated controls. Each concentration of the compound tested was examined in triplicate and the IC_{50} values were determined graphically. The concentrations of compounds used were 5×10^{-5}, 10^{-5}, 10^{-6}, 10^{-7} M. Commercially available Melphalan (Sarcolysin) and Doxorubicin purchased from Arkelan-Glaxo were used as a positive control in the assay.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG №436 RUS 113/905/0-1; RO 362/35-1), program of President of Russian Federation “For Young PhD scientists” (No MK-2464.2008.3), Program of Chemistry and Material Science *Division of RAS*, NIH via the RIMI program (grant 1P20MD001104-01), and NSF/DMR grant 0934212.

References

[1] P.J. Smith, J.R. Dimmock, W.G. Taylor, Can. J. Chem. 50 (1972) 871–879.
[2] P.J. Smith, J.R. Dimmock, W.A. Turner, Can. J. Chem. 51 (1973) 1458–1470.
[3] J.R. Dimmock, N.W. Hamon, K.W. Hindmarsh, A.P. Sellar, W.A. Turner, G.H. Rank, A.J. Robertson, J. Pharm. Sci. 65 (1976) 538–543.
[4] U. Das, R.K. Sharma, J.R. Dimmock, Curr. Med. Chem. 16 (2009) 2001–2020 (and references cited therein).
[5] H.N. Pati, U. Das, R.K. Sharma, J.R. Dimmock, Mini Rev. Med. Chem. 7 (2007) 131–139 (and references cited therein).
[6] H.I. El-Subbagh, S.M. Abu-Zaid, M.A. Mahran, F.A. Badria, A.M. Al-Obaid, J. Med. Chem. 27 (2000) 2915–2921.
[7] M.A. Al-Omar, K.M. Youssef, M.A. El-Sherbeny, A.A. Awadalla, H.I. El-Subbagh, Arch. Pharm. Chem. Life Sci. 338 (2005) 175–180.
[8] J.R. Dimmock, D.W. Elias, M.A. Beazely, N.M. Kandepu, Curr. Med. Chem. 6 (1999) 1125–1149.
[9] J.R. Dimmock, N.M. Kandepu, A.J. Nazarali, T.P. Kowalchuk, N. Motaganahalli, J.W. Quail, P.A. Mykytiuk, G.F. Audette, L. Prasad, P. Perjési, T.M. Allen, C.L. Santos, J. Szydlowski, E. de Clercq, J. Balzarini, J. Med. Chem. 42 (1999) 1358–1366.
[10] E.X. Chen, M.J. Moore, in: H. Kalant, D.M. Grant, J. Mitchell (Eds.), Principles of Medical Pharmacology, seventh ed.. Elsevier Canada, Toronto, 2007 p. 778.
[11] U. Das, S. Das, B. Bandy, J.P. Stables, J.R. Dimmock, Bioorg. Med. Chem. 16 (2008) 3602–3607.
[12] J.R. Dimmock, V.K. Arora, S.L. Wonko, N.W. Hamon, J.W. Quail, Z. Jia, R.C. Warrington, W.D. Fang, J.S. Lee, Drug Des. Deliv. 6 (1990) 183–194.
[13] J.R. Dimmock, R. Kumar, Curr. Med. Chem. 4 (1997) 1–22.
[14] C. Hansch, A. Leo, R.W. Taft, Chem. Rev. 91 (1991) 165–195.
[15] J.R. Dimmock, V.K. Arora, J.W. Quail, U. Pugazhenthi, T.M. Allen, G.Y. Kao, E. De Clercq, J. Pharm. Sci. 83 (1994) 1124–1130.
[16] M.V. Makarov, I.L. Odinets, K.A. Lyssenko, E.Yu. Rybalkina, I.V. Kosilkin, M.Yu. Antipin, T.V. Timofeeva, J. Heterocycl. Chem. 45 (2008) 729–736.
[17] I.L. Odinets, O.I. Artyushin, E.I. Goryunov, K.A. Lyssenko, E.Yu. Rybalkina, I.V. Kosilkin, T.V. Timofeeva, M.Yu. Antipin, Heteroat. Chem. 16 (2005) 497–502.
[18] S. Das, U. Das, P. Selvakumar, R.K. Sharma, J. Balzarini, E. De Clercq, J. Molnár, J. Serly, Z. Baráth, G. Schatte, B. Bandy, D.K.J. Gorecki, J.R. Dimmock, ChemMedChem 4 (2009) 1831–1840.
[19] M.V. Makarov, E.S. Leonova, E.Yu. Rybalkina, P. Tongwa, V.N. Khrustalev, T.V. Timofeeva, I.L. Odinets, Eur. J. Med. Chem. 45 (2010) 992–1000.
[20] M.V. Makarov, E.Yu. Rybalkina, G.-V. Röschenthaler, K.W. Short, T.V. Timofeeva, I.L. Odinets, Eur. J. Med. Chem. 44 (2009) 2135–2144.
[21] E. Leonova, M. Makarov, Z. Klemenkova, I. Odinets, Helv. Chim. Acta (2010). doi:10.1002/hlca.201000005.
[22] S.Z. Vatsadze, M.A. Manaenkova, N.V. Sviridenkova, N.V. Zyk, D.P. Krut'ko, A.V. Churakov, M.Yu. Antipin, J.A.K. Howard, H. Lang, Russ. Chem. Bull. 55 (2006) 1184–1194.
[23] S.G. Aller, J. Yu, A. Ward, Y. Weng, S. Chittaboina, R. Zhuo, P.M. Harrell, Y.T. Trinh, Q. Zhang, I.L. Urbatsch, G. Chang, Science 323 (2009) 1718–1722.
[24] R.F. Hudson, L. Keay, J. Chem. Soc. (1956) 2463–2469.
[25] L.F. Tomchina, P.M. Zavlin, V.V. Razumovskii, Russ. J. Gen. Chem. 38 (1968) 549–566.
[26] G.M. Sheldrick, SADABS, V. 2.03, Bruker/Siemens Area Detector Absorption Correction Program. Bruker AXS, Wisconsin, Madison, 2003.
[27] G.M. Sheldrick, Acta Crystallogr. A64 (2008) 112–122.