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Design, antiviral and cytostatic properties of isoxazolidine-containing amonafide analogues

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ABSTRACT

A novel series of 5-arylcarbamoyl- and 5-arylmethyl-2-methylisoxazolidin-3-yl-3-phosphonates have been synthesized via cycloaddition of *N*-methyl-*C*-(diethoxyphosphoryl)nitrone with *N*-substituted naphthalimide acrylamides and *N*-allylnaphthalimides. All *cis*- and *trans*-isoxazolidine phosphonates obtained herein were assessed for antiviral activity against a broad range of DNA and RNA viruses. Isoxazolidines *trans*-**9d** and *trans*-**9f** exhibited the highest activity (EC_{50} = 8.9 μM) toward cytomegalovirus. Compounds *cis*- and *trans*-**9d** as well as *cis*- and *trans*-**9f** were found potent against HSV and Vaccinia viruses (EC_{50} in the 45–58 μM range), whereas isoxazolidines **10a** and **10d** suppressed replication of Coxsackie B4 and Punta Toro viruses (EC_{50} in the 45–73 μM range). Antiproliferative evaluation of all obtained isoxazolidines revealed the promising activity of *cis*-**9b**, *cis*-**9d**, *trans*-**9d**, *cis*-**9e**, *trans*-**9e**, *cis*-**9f** and *trans*-**9f** toward tested cancer cell lines with IC_{50} in the 1.1–19 μM range.

1. Introduction

Despite significant achievements in cancer chemotherapy a search for more selective compounds with capability to better differentiate malignant tumors from normal cells and able to minimize side effects of current drugs continues. Taking into account their mechanisms of action, therapeutic agents belong to different pharmacological classes. Among them, those which interact with DNA, such as DNA alkylating agents, intercalators or groove binders, are of special interest.[1–3] Intercalators constitute a well explored class of compounds because their mode of action is the most predictable and relies on inhibition of replication process by reversible binding to DNA.[4,5] The planar aromatic fragments of intercalators insert into the DNA double helix, thereby distorting the DNA backbone conformation and poison the DNA topoisomerases I or II.[6] Several factors are responsible for stabilization a drug–DNA complex, namely stacking π-bond interactions, van der Waals forces and eventually hydrogen bonding between the aromatic fragment of a drug moiety and the purine/pyrimidine bases present in DNA strains. Intercalators can be classified according to their aromatic/heteroaromatic subunit or electrostatic potential they posses.[2,3] Among them, naphthalimide derivatives have been found promising as anticancer agents since they are able to intercalate into DNA and some of them, including amonafide **1**[7,8] and mitonafide **2**,[9] have reached clinical trials for treatment of solid tumors, however most of them showed poor therapeutic indices.

On the other hand, various functional groups present in the structure of intercalators may also contribute to the binding of a drug to DNA. The amino group of a sugar residue in doxorubicin **3** forms an ionic bond with the phosphate function of the DNA which results in additional and very efficient stabilization of locking the drug into an active site.[10] There are also known groups of intercalators which activities are related to the specific shape of molecule rather than their aromatic ring system. Studies on intercalating properties of 2-[(arylmethyl)amino]-2-methyl-1,3-propanodiols (AMAPs) **4** proved that the amino side chain is responsible for DNA binding due to electrostatic interactions and enhanced activity which was observed for derivatives with 2-amino-1,3-propanediol moiety (Fig. 1).[11,12]

Compounds **5** (Fig. 2) have been designed as new isoxazolidine-containing intercalators equipped with planar polycyclic aromatic frameworks at C3 and their cytotoxic and apoptotic properties have already been described.[13,14] Recently, we succeeded in the preparation of compounds **6** having 1- and 2-naphthyl substituents at C5 of an isoxazolidine ring and they proved cytotoxic against HeLa and K562 cell lines with IC_{50} values 50 and 90 μM, respectively.[15] Moreover, isoxazolidines **7** containing at C5 a carbamoyl linker which separates the isoxazolidine ring and substituted phenyls suppressed divisions of three cancer cell lines at concentrations ranging from 228 to 102 μM.[16]

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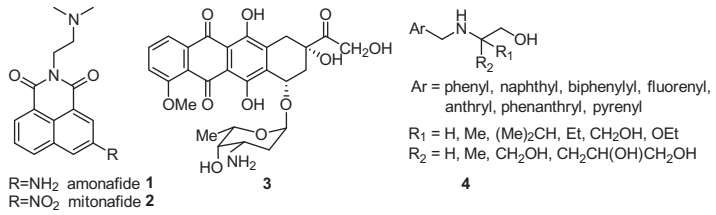


Figure 1. Structures of known intercalators.

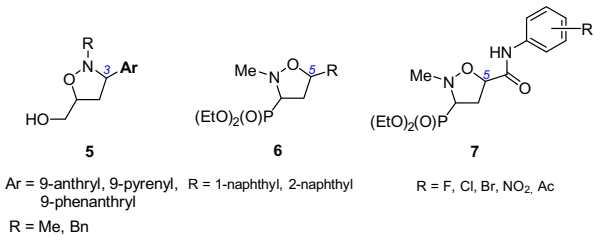


Figure 2. Biologically active isoxazolidines.

Herein, a new series of 5-arylcarbamoyl- and 5-arylmethylisoxazolidines substituted at C3 with a dialkoxyphosphoryl functionality **9** and **10** have been designed as a continuation of an ongoing project directed towards the construction of compounds with antineoplastic activity based on application of the phosphoryl nitrone **8** (Scheme 1).

An idea behind designing compounds **9** and **10** is based on the combination of two biologically active moieties/pharmacophores, namely naphthalimide and (3-diethoxyphosphoryl)isoxazolidine units, with intention to obtain their active hybrids or conjugated drugs.[17] Recently, similar approach was successfully applied by several research groups and resulted in synthesizing various series of amonafide-containing chimeras,[18] including naphthalimides conjugated with carbazole,[19] benzodiazepine,[20–22] benzoic acid,[23] aliphatic diamine chain[24] as well as peptide nucleic acid.[25] Our approach to the synthesis of phosphonylated intercalators **9** and **10** relies on the assumption that the designed molecules contain both aromatic systems able to intercalate into DNA and the diethoxyphosphoryl function at C3 of the isoxazolidine ring which could be further phosphorylated but also contains a non-hydrolysable C–P bond.[26]

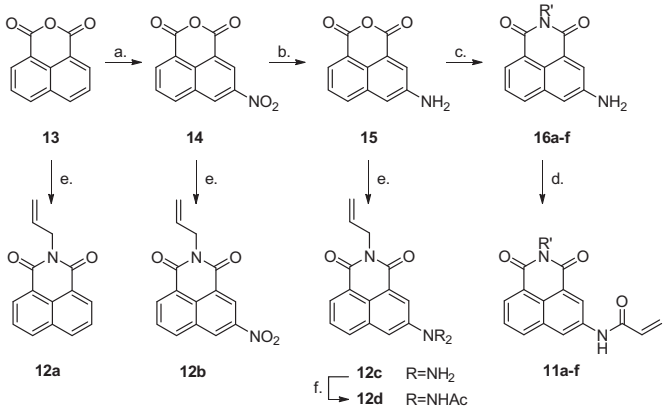
2. Results and discussion

2.1. Chemistry

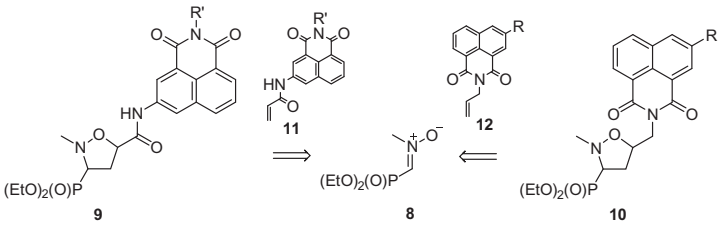
To the best of our knowledge all acrylamides **11** as well as allylated naphthalimides **12**, except for **12a** and **12b** have not been described in the literature. For the purpose of this study, *N*-substituted acrylamides **11** were synthesized starting from commercially available naphthalic anhydride **13** via multistep procedures including nitration[27] followed by a standard reduction of the nitro group,[28] transformation of the aminoanhydride **15** into naphthalimides **16a–f** by treatment with alkyl amines or ethylenediamines[29] and finally preparation of acrylamides **11a–f** from **16a–f** and acryloyl chloride in the presence of triethylamine (Scheme 2). The series of dipolarophiles **12a–c** were obtained in the reaction of naphthalic anhydrides **13**, **14** and **15** with allylamine.[29] Furthermore, naphthalimide **12c** was converted into its acetyl derivative **12d**.[30]

1,3-Dipolar cycloadditions of a nitrone **8**[31] with naphthalimides **11a–f** were performed in toluene or a toluene–chloroform mixture at 70 °C and led to the formation of diastereoisomeric mixtures of (3-diethoxyphosphoryl)isoxazolidines *trans*-**9a–f** and *cis*-**9a–f** (Scheme 3; Table 1). In all cases moderate *trans*/*cis* diastereoselectivities were observed with *trans*-isomers **9a–f** predominating (de 56–72%). Purification of the crude mixtures of cycloadducts on silica gel columns resulted in separation of all major diastereoisomers *trans*-**9a–f** as well as all minor isomers *cis*-**9a–f**.

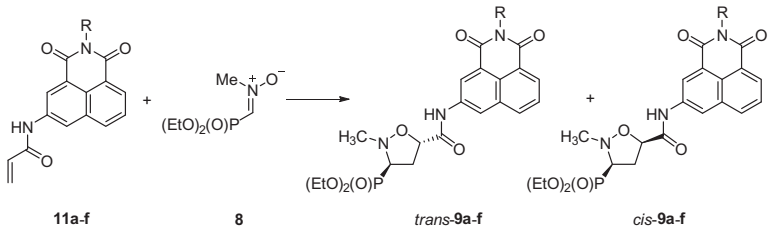
Reactions of a nitrone **8** with *N*-allylated naphthalimides **12a–d** were carried out in toluene or toluene–chloroform solutions at 70 °C and gave mixtures of isomeric isoxazolidines *trans*-**10a–d** and *cis*-**10a–d** with good *trans*/*cis* diastereoselectivities (de 72–82%). Crude mixtures of cycloadducts were subjected to purification on silica gel columns and subsequent crystallization.



Scheme 2. Reaction conditions: (a) H_2SO_4, HNO_3; (b) $SnCl_2$, HCl; (c) R′NH_2, EtOH, see Table 1; (d) acryloyl chloride, NEt_3, see Table 1; (e) allylamine, EtOH; (f) Ac_2O.

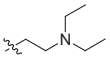
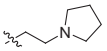


Scheme 1. Retrosynthesis of (isoxazolidinyl)phosphonates **9** and **10**.



Scheme 3. Reaction conditions: toluene or toluene–chloroform, 70 °C.

Table 1
Isoxazolidines *trans*-**9** and *cis*-**9** obtained according to Scheme 3

Entry	Acrylamide **11** R	Ratio of *trans*-**9**/*cis*-**9**	Yield (%)
A		81:19	*trans*-**9a** (63)[a] + *cis*-**9a** (11)[a] + *trans*-**9a** and *cis*-**9a** (11)[b]
B		84:16	*trans*-**9b** (71)[a] + *cis*-**9b** (14)[a] + *trans*-**9b** and *cis*-**9b** (2)[b]
C		79:21	*trans*-**9c** (67)[a] + *cis*-**9c** (19)[a] + *trans*-**9c** and *cis*-**9c** (5)[b]
D		79:21	*trans*-**9d** (33)[a] + *cis*-**9d** (8)[a] + *trans*-**9d** and *cis*-**9d** (52)[b]
E		86:14	*trans*-**9e** (32)[a] + *cis*-**9e** (11)[a] + *trans*-**9e** and *cis*-**9e** (54)[b]
F		78:22	*trans*-**9f** (31)[a] + *cis*-**9f** (8)[a] + *trans*-**9f** and *cis*-**9f** (50)[b]

[a] Yield of pure isomer.
[b] Yield of pure mixture of *cis*- and *trans*-isomers.

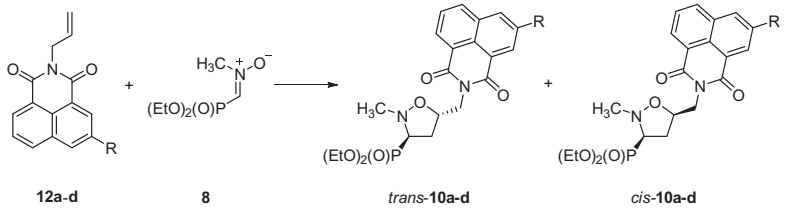
However, attempts at separating diastereoisomers *trans*-**10a–d** and *cis*-**10a–d** failed leading to mixtures enriched in isomers *trans*-**10** (Scheme 4; Table 2).

The relative configurations of the isoxazolidines *trans*-**9a–f** and *cis*-**9a–f** were determined taking advantage of our previous studies on stereochemistry of cycloaddition of *N*-methyl-*C*-diethoxyphosphorylnitrone **8** with (hetero)arylacrylamides[16] since similar ^{1}H NMR spectral patterns for the respective series of isoxazolidines *trans*-**9a–f** and *cis*-**9a–f** were observed as compared to the previously described *trans*- and *cis*-5-(hetero)arylcarbamoyl-2-methylisoxazolidin-3-yl-3-phosphonates.[16,32] Briefly, for the major *trans*-isomers **9a–f** the $_3E$ conformation (Fig. 3) of an isoxazolidine ring was established taking advantage of the diagnostic values of vicinal coupling constants [J_{CCCP} = 7.7–8.9 Hz, $J_{H3-H4\alpha}$ = 8.1–8.3 Hz, $J_{H3-H4\beta}$ = 8.5–8.9 Hz, $J_{H4\alpha-P}$ = 9.4–10.3 Hz, $J_{H4\beta-P}$ = 15.7–16.1 Hz, $J_{H4\alpha-H5}$ = 5.5–5.8 Hz and $J_{H4\beta-H5}$ = 8.7–8.9 Hz] extracted from the respective ^{1}H and ^{13}C NMR spectra. In this conformation the diethoxyphosphoryl group resides in the equatorial position of the isoxazolidine ring while carbamoyl substituents are located pseudoequatorially. Similarly, relative configurations of isoxazolidines *trans*-**10a–d** and *cis*-**10a–d** were established based on very close stereochemical outcomes found for the reaction between a nitrone **8** and *N*-allylated naphthalimides **12a–d** as compared with already elaborated analogous reactions of a nitrone **8** and *N*-allylated nucleobases.[33]

2.2. Antiviral and cytostatic evaluation

2.2.1. Antiviral activity

Pure 5-arylcarbamoyl-2-methylisoxazolidin-3-yl-3-phosphonates *trans*-**9a–f** and *cis*-**9a–f** and inseparable mixtures of 5-arylmethyl-2-methylisoxazolidin-3-yl-3-phosphonates *trans*-**10a–**



Scheme 4. Reaction conditions: toluene or toluene–chloroform, 70 °C.

Table 2
Isoxazolidines *trans*-**10** and *cis*-**10** obtained according to Scheme 4

Entry	Imide **9** R	Ratio of *trans*-**10**/*cis*-**10**	Yield[a] (%)
A	H	91:9	*trans*-**10a** and *cis*-**10a** (76%; *trans*-**10a**/*cis*-**10a** = 93:7)
B	NO_2	91:9	*trans*-**10b** and *cis*-**10b** (67%; *trans*-**10b**/*cis*-**10b** = 94:6)
C	NH_2	86:14	*trans*-**10c** and *cis*-**10c** (32%; *trans*-**10c**/*cis*-**10c** = 93:7)
D	$NHC(O)CH_3$	88:12	*trans*-**10d** and *cis*-**10d** (73%; *trans*-**10d**/*cis*-**10d** = 93:7)

[a] Yield of pure mixture of *cis*- and *trans*-isomers.

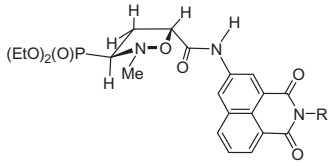


Figure 3. The $_3E$ conformation of an isoxazolidine ring in *trans*-**9a**–**f**.

d/*cis*-**10a**–**d** were evaluated for inhibitory activity against a wide variety of DNA and RNA viruses, using the following cell-based assays: (a) human embryonic lung (HEL) cells: herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), thymidine kinase deficient (acyclovir resistant) herpes simplex virus-1 (TK$^-$ KOS ACV^r), vaccinia virus, vesicular stomatitis virus and, adenovirus-2, cytomegalovirus (AD-169 strain and Davis strain), varicella-zoster virus (TK$^+$ VZV strain and TK$^-$ VZV strain); (b) HeLa cell cultures: vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus; (c) Vero cell cultures: *para*-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus; (d) MDCK cell cultures: influenza A virus (H1N1 and H3N2 subtypes) and influenza B virus and (e) CrFK cell cultures: feline herpes virus (FHV) and feline corona virus (FIPV). Ganciclovir, cidofovir, acyclovir, brivudin, (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], oseltamivir carboxylate, amantadine, rimantadine, ribavirin, dextran sulfate (molecular weight 5000, DS-5000), Hippeastrum hybrid agglutinin (HHA) and Urtica dioica agglutinin (UDA) were used as the reference compounds. The antiviral activity was expressed as the EC_{50}: the compound concentration required to reduce virus plaque formation (VZV) by 50% or to reduce virus-induced cytopathogenicity by 50% (other viruses).

Some compounds of the series of isoxazolidines *cis*-**9**/*trans*-**9** were found active against human cytomegalovirus with activities below 60 μM (Table 3). Among them, *trans*-**9d** (EC_{50} = 8.9 μM) and *trans*-**9f** (EC_{50} = 8.9–20 μM) exhibited the highest activity with EC_{50} values comparable to those found for the reference compound ganciclovir which is also an approved drug for HMCV, and approximately an order of magnitude lower than that assayed for cidofovir used as the second reference. However, the new-generation drug letermovir showed EC_{50} = 0.0046 ± 0.0019 μM against HCMV in AD169 strain and recently reached phase IIb of clinical trials.[34]

Among the series of isoxazolidines *cis*-**9**/*trans*-**9**, majority of the derivatives was able to inhibit replication of TK$^+$ and TK$^-$ VZV strains with EC_{50} values in the range of 20–45 μM (Table 4). The potency against TK$^+$ VZV strain of the most active *trans*-**9d** (EC_{50} = 14–15 μM) was found an order or three orders of magnitude lower than that of the reference acyclovir (also the approved drug) or brivudin, respectively. However, activities of isoxazolidines studied (except *trans*-**9c**) against the TK$^-$ VZV strain compare favorably (EC_{50} = 20–45 μM) with that of the reference acyclovir (EC_{50} = 33–44 μM) being an order of magnitude less active than the second reference brivudin (EC_{50} = 1.0–4.9 μM).

On the other hand, none of the compounds from the series *trans*-**10**/*cis*-**10** was found active either towards HCMV or against VZV.

Although the isoxazolidine derivatives equipped with *N*,*N*-dimethylaminoethyl (*trans*-**9d** and *cis*-**9d**) and 2-(pirolidyn-1-yl)ethyl (*trans*-**9f**) functions were found slightly active against three strains of herpes simplex virus with EC_{50} values ranging from 45 to 100 μM (Table 5) their potency seems marginal when compared with that of the four reference compounds (brivudin, cidofovir, ganciclovir and the FDA-approved acyclovir). However, activities of *trans*-**9d**, *cis*-**9d**, *trans*-**9f** and *cis*-**9f** against vaccinia virus (EC_{50} = 45–100 μM) are of considerable value since the reference acyclovir and ganciclovir were inactive while the two other reference compounds brivudin and cidofovir showed EC_{50} values of 17–22 and 22 μM (Table 5), respectively. Moreover, these compounds were not cytotoxic toward HEL cells (in which the viruses were replicated) at concentrations up to 100 μM or displayed a minimum cytotoxic concentration (MCC) of 100 μM.

In the second series of isoxazolidine phosphonates **10**, *trans*-**10a**/*cis*-**10a** and *trans*-**10d**/*cis*-**10d** inhibited the replication of Coxsackie virus B4 at concentrations 45–73 μM which was significantly better than ribavirin (Table 6). On the other hand, only *trans*-**10d**/*cis*-**10d** showed a weak activity against Punta Toro virus (EC_{50} = 50–100 μM) which again was higher than that of ribavirin (Table 6). These compounds did not alter morphology of Vero cells where the antiviral assays were performed. Since no drugs for Coxackie and Punta Toro viruses have been approved so far, the urgency of search for active compounds is justified.

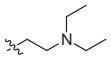
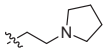
None of the isoxazolidines *cis*-**9**/*trans*-**9** and *trans*-**10** and *cis*-**10** phosphonates showed activity against the other tested viruses,

Table 3
Cytotoxicity and antiviral activity against cytomegalovirus in HEL cell cultures

Compound	R	Antiviral activity EC_{50}[a] (μM)		Cytotoxicity (μM)
		AD-169 strain	Davis strain	Cell morphology MCC[b]
cis-**9a**				
(exp. 1)		45	45	>100
(exp. 2)		45	45	>100
trans-**9a**				
(exp. 1)		>20	55	>100
(exp. 2)		49	45	>100
cis-**9b**				
(exp. 1)		>20	>20	100
(exp. 2)		>20	>20	100
trans-**9b**				
(exp. 1)		>20	>20	100
(exp. 2)		>20	>20	100
cis-**9c**				
(exp. 1)		45	45	>100
(exp. 2)		45	45	>100
trans-**9c**				
(exp. 1)		>100	>100	>100
(exp. 2)		>100	>100	>100
cis-**9d**				
(exp. 1)		45	41	>100
(exp. 2)		37	37	>100
trans-**9d**				
(exp. 1)		8.9	8.9	100
(exp. 2)		8.9	8.9	100
cis-**9e**				
(exp. 1)		100	100	>100
(exp. 2)		100	>100	>100
trans-**9e**				
(exp. 1)		55	49	>100
(exp. 2)		55	63	>100
cis-**9f**				
(exp. 1)		45	45	>100
(exp. 2)		45	45	>100
trans-**9f**				
(exp. 1)		8.9	10.9	100
(exp. 2)		10.9	20	100
Cidofovir				
(exp. 1)		0.92	1.02	>350
(exp. 2)		0.92	1.14	>350
Ganciclovir				
(exp. 1)		7.1	7.1	>350
(exp. 2)		6.3	7.9	>350

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alternation of cell morphology.

Table 4
Cytotoxicity and antiviral activity against varicella-zoster virus (VZV) in HEL cell cultures

Compound	R	Antiviral activity EC_{50}[a] (μM)		Cytotoxicity (μM)
		TK^+ VZV strain	TK^- VZV strain	Cell morphology MCC[b]
cis-**9a**				
(exp. 1)		20	38	>100
(exp. 2)		32	45	>100
trans-**9a**				
(exp. 1)		32	40	>100
(exp. 2)		28	41	>100
cis-**9b**				
(exp. 1)		20	38	>100
(exp. 2)		32	45	>100
trans-**9b**				
(exp. 1)		32	40	>100
(exp. 2)		28	41	>100
cis-**9c**				
(exp. 1)		45	45	>100
(exp. 2)		45	45	>100
trans-**9c**				
(exp. 1)		>100	>100	>100
(exp. 2)		>100	>100	>100
cis-**9d**				
(exp. 1)		>20	20	100
(exp. 2)		11	20	100
trans-**9d**				
(exp. 1)		14	26	>100
(exp. 2)		15	34	>100
cis-**9e**				
(exp. 1)		>20	36	>100
(exp. 2)		41	44	>100
trans-**9e**				
(exp. 1)		30	32	100
(exp. 2)		>20	>20	100
cis-**9f**				
(exp. 1)		>20	>20	100
(exp. 2)		>20	79	>100
trans-**9f**				
(exp. 1)		>20	>20	100
(exp. 2)		>20	>20	100
Acyclovir				
(exp. 1)		3.6	44	>440
(exp. 2)		1.5	33	>440
Brivudin				
(exp. 1)		0.042	1.0	>300
(exp. 2)		0.029	4.9	>300

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alternation of cell morphology.

including adenovirus-2, vesicular stomatitis virus, respiratory syncytial virus, *para*-influenza-3 virus, reovirus-1, Sindbis virus, influenza A and B viruses, feline herpes virus, and feline corona virus.

2.2.2. Cytostatic activity

The 50% cytostatic inhibitory concentration (IC_{50}) causing a 50% decrease in cell proliferation was determined against murine leukemia L1210, human lymphocyte CEM and human cervix carcinoma HeLa cells. The synthesized compounds showed differences in their antiproliferative activity with IC_{50} ranging from 1.1 to 180 μM (Table 7). Among isoxazolidines having a carbamoyl linker, structure–activity relationship studies indicated that the presence of a tertiary nitrogen atom in a side chain significantly increased cytostatic activity of the tested compounds. Phosphonates *trans*-**9a–c** and *cis*-**9a–c** having straight aliphatic chains were considerably less cytostatic (IC_{50} = 20–85 μM) than those containing tertiary amino functions, namely *trans*-**9d–f** and *cis*-**9d–f**. Moreover, compounds *trans*-**9a** and *cis*-**9c** decreased viability of the MDCK cell line at concentrations of 46.2 and 51.8 μM (CC_{50}), respectively. Furthermore, both *trans*- and *cis*-isoxazolidines **9d**, **9e** and **9f** having aminoalkyl substituents appeared to be the most cytostatic towards L1210, CEM and HeLa cell lines with IC_{50}'s below 23 μM. Among them, *trans*-**9d** and *cis*-**9d**, which can be considered as amonafide analogues, as well as *trans*-**9f** and *trans*-**9f** proved highly cytostatic toward murine leukemia L1210 and human cervix carcinoma HeLa cells (IC_{50} = 1.1–3.3 μM) and their potency was only an order of magnitude lower than that of a reference 5-fluorouracil. Besides their high cytostatic activity, compounds *trans*-**9d**, *cis*-**9d**, *trans*-**9f** and *cis*-**9f** were found to reduce viability of MDCK and CrFK cells at concentrations in the range of 95.2–62.2 μM (CC_{50}). In addition, phosphonates *trans*-**9a**, *trans*-**9b**, *cis*-**9b**, *cis*-**9c**, *trans*-**9e** and *cis*-**9e** exhibited high cytostatic activity against human lymphocyte cells (CEM), comparable to that of 5-fluorouracil, while *trans*-**9d** and *trans*-**9f** were even five-fold more active than 5-fluorouracil. Furthermore, although no correlation between configuration of isoxazolidines *trans*-**9a–c**/*cis*-**9a–c** and *trans*-**9e**/*cis*-**9e** and their biological activity was observed, *trans*-configured isoxazolidines *trans*-**9d** and *trans*-**9f** were found two-fold more cytostatic than the corresponding *cis*-isomers *cis*-**9d** and *cis*-**9f**.

The second series of 5-arylmethyl-2-methylisoxazolidin-3-yl-3-phosphonates *trans*-**10**/*cis*-**10** also showed considerable cytostatic activity against all three tested cancer cell lines (IC_{50} from 20 to 180 μM). Isoxazolidines *trans*-**10b**/*cis*-**10b** substituted with the nitro group at C5 of a naphthalimide unit emerged as the most cytostatic with the highest potency toward HeLa cell line (IC_{50} = 20 μM). None of the evaluated compounds *trans*-**10**/*cis*-**10** decreased viability of MDCK and CrFK cells at concentration up to 100 μM.

3. Conclusions

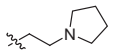
Two new series of 5-arylcarbamoyl-2-methylisoxazolidin-3-yl-3-phosphonates *trans*-**9** and *cis*-**9** and 5-arylmethyl-2-methylisoxazolidin-3-yl-3-phosphonates *trans*-**10** and *cis*-**10** have been obtained from *N*-methyl-*C*-(diethoxyphosphoryl)nitrone **8** and the respective *N*-substituted naphthalimide acrylamides or *N*-allylated naphthalimides via the 1,3-dipolar cycloaddition.

All synthesized isoxazolidine phosphonates *trans*-**9** and *cis*-**9** as well as the respective mixtures of *trans*-**10**/*cis*-**10** were evaluated against a variety of DNA and RNA viruses. Several of these derivatives showed some activity against varicella-zoster virus and cytomegalovirus. Among all tested compounds, isoxazolidines *trans*-**9d** and *trans*-**9f** exhibited the highest activity (EC_{50} = 8.9 μM) toward cytomegalovirus, comparable to the activity of ganciclovir, the approved drug which was used as the reference compound. The isoxazolidines **9** (except *trans*-**9c**) appeared active against the TK^- VZV strain and the potency of compound *cis*-**9d** (EC_{50} = 20 μM) compares favorably with that of the reference acyclovir (EC_{50} = 33–44 μM) which is also the approved drug.

Some of the tested compounds were also endowed with the antiviral activity against HSV and Vaccinia (*cis*- and *trans*-**9d**, *cis*- and *trans*-**9f**, EC_{50} in the 45–58 μM range), Coxsackie B4 and Punta Toro (**10a** and **10d**, EC_{50} in the 45–73 μM range) viruses and although for the Coxsackie virus B4 compounds **10a** and **10d** are even more active than ribavirin used as the reference compound their potency is not large enough to deserve further studies.

Cytostatic activity of *trans*-**9**, *cis*-**9** and *trans*-**10**/*cis*-**10** was evaluated on L1210, CEM and HeLa cell lines and for the most cytostatic compounds (*cis*-**9b**, *cis*-**9d**, *trans*-**9d**, *cis*-**9e**, *trans*-**9e**, *cis*-**9f** and *trans*-**9f**), IC_{50} values were found in the 1.1–12 μM range.

Table 5
Cytotoxicity and antiviral activity in HEL cell cultures

Compound	R	MCC[a] (μM)	EC_{50}[b] (μM)			
			HSV-1 (KOS)	HSV-1 (TK^- KOS ACV^r)	HSV-2 (G)	Vaccinia virus
trans-**9d**						
(exp. 1)		⩾100	45	45	45	45
(exp. 2)		⩾100	45	45	>100	>100
cis-**9d**						
(exp. 1)		>100	50	50	50	45
(exp. 2)		>100	58	58	58	>100
trans-**9f**						
(exp. 1)		⩾100	45	45	45	45
(exp. 2)		⩾100	45	45	>100	>100
cis-**9f**						
(exp. 1)		>100	>100	>100	58	50
(exp. 2)		>100	>100	>100	100	58
Brivudin						
(exp. 1)		>250	0.01	2.0	146	17
(exp. 2)		>250	0.05	10	250	22
Cidofovir						
(exp. 1)		>250	1.0	1.2	1.0	22
(exp. 2)		>250	2.0	2.0	1.5	22
Acyclovir						
(exp. 1)		>250	0.2	2.0	0.08	>250
(exp. 2)		>250	0.2	2.0	0.2	>250
Ganciclovir						
(exp. 1)		>100	0.03	0.8	0.03	>100
(exp. 2)		>100	0.02	0.5	0.03	>100

[a] Minimum cytotoxic concentration that causes a microscopically detectable alternation of cell morphology.
[b] Effective concentration that is required to reduce virus-induced cytopathogenicity by 50%.

Table 6
Cytotoxicity and antiviral activity in Vero cell cultures

Compound	R	MCC[a] (μM)	EC_{50}[b] (μM)	
			Coxsackie virus B4	Punta Toro virus
trans-**10a**/*cis*-**10a** (93:7)				
(exp. 1)	H	>100	45	>100
(exp. 2)		>100	73	>100
trans-**10d**/*cis*-**10d** *(93:7)*				
(exp. 1)	$NHC(O)CH_3$	>100	45	50
(exp. 2)		>100	58	100
DS-10.000 (μg/ml)				
(exp. 1)		>100	>100	45
(exp. 2)		>100	>100	100
Ribavirin				
(exp. 1)		>250	>250	112
(exp. 2)		>250	>250	250

[a] Minimum cytotoxic concentration that causes a microscopically detectable alternation of cell morphology.
[b] Effective concentration that is required to reduce virus-induced cytopathogenicity by 50%.

Generally, compounds with a carbamoyl linker *trans*-**9**/*cis*-**9** were more active than *trans*-**10**/*cis*-**10** having a methylene bridge.

4. Experimental section

^1H NMR spectra were taken in $CDCl_3$ on the following spectrometers: Varian Mercury-300 and Bruker Avance III (600 MHz) with TMS as internal standard. ^{13}C NMR spectra were recorded for $CDCl_3$ solution on the Varian Mercur-300 machine at 75.5 MHz, while for DMSO solution on Bruker Avance III at 151.0 MHz. ^{31}P NMR spectra were performed in $CDCl_3$ solution on the Varian Mercury-300 at 121.5 MHz or on Bruker Avance III at 243.0 MHz. IR spectra were measured on an Infinity MI-60 FT-IR spectrometer. Melting points were determined on Boetius apparatus and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory of this Faculty on Perkin–Elmer PE 2400 CHNS analyzer. The following adsorbents were used: column chromatography, Merck silica gel 60 (70–230 mesh); analytical TLC, Merck TLC plastic sheets silica gel 60 F_{254}.

4.1. General procedure for the preparation of 1,8-naphthalimides 16a–f and 12a–c

A suspension of 1,8-naphthalic anhydride **13**, **14** or **15** (1.00 mmol), appropriate aliphatic amine, substituted ethylenediamine or allylamine (2.00 mmol) in ethanol (10 mL) was heated under reflux for 3 h. After evaporation of a solvent under reduced pressure the crude product was purified on a silica gel column with chloroform/methanol mixtures (100:1, 50:1 v/v) to give the corresponding 1,8-naphthalimides **16a–f** and **12a–c**.

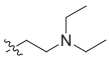
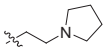
4.1.1. *N*-Propyl-3-amino-1,8-naphthalimide (16a)

Yield: 74%; yellow amorphous solid (crystallized from chloroform/hexane) mp 202–203 °C; IR (KBr, cm^{-1}) ν_{max}: 3473, 3368, 1688, 1646, 1580, 1341, 1308, 1220, 778, 744; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.31 (dd, 1H, J = 7.3, 1.1 Hz), 8.02 (d, 1H, J = 2.3 Hz), 7.92 (dd, 1H, J = 8.3, 1.1 Hz), 7.60 (dd, 1H, J = 8.3, 7.3 Hz), 7.30 (d, 1H, J = 2.3 Hz), 4.18 (br s, 2H, NH_2), 4.13 (d, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 1.81–1.69 (m, 2H, $CH_2CH_2CH_3$), 1.01 (d, 3H, J = 7.5 Hz, $CH_2CH_2CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 164.51 (s, C(O)), 164.28 (s, C(O)), 145.33, 133.50, 131.71, 127.54, 127.30, 123.79, 122.67, 122.62, 122.11, 114.05, 42.18 (s, $CH_2CH_2CH_3$), 21.71 (s, $CH_2CH_2CH_3$), 11.85 (s, $CH_2CH_2CH_3$). Anal. Calcd for $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N, 11.02; found: C, 70.61; H, 5.70; N, 10.77.

4.1.2. *N*-Isobutyl-3-amino-1,8-naphthalimide (16c)

Yield: 77%; yellow amorphous solid (crystallized from chloroform/hexane) mp 185–186 °C; IR (KBr, cm^{-1}) ν_{max}: 3452, 3357,

Table 7
Inhibitory effect of the tested compounds against the proliferation of murine leukemia (L1210), human T-lymphocyte (CEM) and human cervix carcinoma cells (HeLa)

Compound	R	IC_{50}[a] (μM)			CC_{50}[b] (μM)	
		L1210	CEM	HeLa	MDCK	CrFK
cis-**9a**		76 ± 16	47 ± 18	70 ± 41	>100	>100
trans-**9a**		41 ± 7	28 ± 4	74 ± 11	46.2	>100
cis-**9b**		28 ± 7	19 ± 1	25 ± 3	>100	>100
trans-**9b**		85 ± 54	25 ± 7	78 ± 3	>100	>100
cis-**9c**		73 ± 27	26 ± 3	49 ± 21	51.8	>100
trans-**9c**		72 ± 52	48 ± 10	56 ± 27	>100	>100
cis-**9d**		2.9 ± 0.2	6.0 ± 1.1	3.3 ± 0.3	>100	62.2
trans-**9d**		1.5 ± 0.3	3.4 ± 0.4	1.8 ± 0.9	75.2	95.2
cis-**9e**		16 ± 4	23 ± 0	7.3 ± 2.4	>100	>100
trans-**9e**		11 ± 0	21 ± 1	13 ± 5	>100	>100
cis-**9f**		4.4 ± 0.1	12 ± 1	3.0 ± 0.6	>100	89.1
trans-**9f**		1.7 ± 0.4	3.9 ± 0.0	1.1 ± 0.0	87.3	>100
trans-**10a**/*cis*-**10a** (93:7)	H	180 ± 47	111 ± 14	77 ± 20	>100	>100
trans-**10b**/*cis*-**10b** (94:6)	NO_2	34 ± 6	51 ± 6	20 ± 3	>100	>100
trans-**10c**/*cis*-**10c** (93:7)	NH_2	95 ± 42	99 ± 2	101 ± 58	>100	>100
trans-**10d**/*cis*-**10d** (93:7)	$NHC(O)CH_3$	136 ± 14	125 ± 4	111 ± 3	>100	>100
5-Fluorouracil		0.33 ± 0.17	18 ± 5	0.54 ± 0.12	—	—

[a] 50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.
[b] 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

1692, 1657, 1627, 1581, 1447, 1343, 1297, 1222, 1072, 783; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.33 (dd, 1H, J = 7.3, 1.0 Hz), 8.03 (d, 1H, J = 2.4 Hz), 7.93 (dd, 1H, J = 8.3, 1.0 Hz), 7.60 (dd, 1H, J = 8.3, 7.3 Hz), 7.31 (d, 1H, J = 2.4 Hz), 4.15 (br s, 2H, NH_2), 4.03 (d, 2H, J = 7.3 Hz, CH_2CH$(CH_3)_2$), 2.28–2.18 (m, 1H, $CH_2CH(CH_3)_2$), 0.98 (d, 6H, J = 6.7 Hz, $CH_2CH(CH_3)_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 164.79 (s, C(O)), 164.57 (s, C(O)), 145.33, 133.50, 131.67, 127.61, 127.31, 123.75, 122.72, 122.59, 122.21, 114.05, 47.40 (s, CH_2CH$(CH_3)_2$), 27.69 (s, CH_2C$H(CH_3)_2$), 20.62 (s, $CH_2CH(CH_3)_2$). Anal. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44; found: C, 71.42; H, 5.84; N, 10.19.

4.1.3. *N*-(2-Propenyl)-3-amino-1,8-naphtalimide (12c)

Yield: 92%; orange amorphous solid mp 221–222 °C; IR (KBr, cm^{-1}) ν_{max}: 3455, 3364, 1688, 1644, 1615, 1578, 1448, 1379, 1330, 1237, 1180, 777, 744; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.32 (dd, 1H, J = 7.3, 1.0 Hz), 8.03 (d, 1H, J = 2.4 Hz), 7.93 (dd, 1H, J = 8.1, 1.0 Hz), 7.60 (dd, 1H, J = 8.1, 7.3 Hz), 7.30 (d, 1H, J = 2.4 Hz), 5.99 (ddt, 1H, J = 17.0, 10.3, 5.7 Hz, CH_2CH=CH_2), 5.31 (dq, 1H, J = 17.0, 1.3 Hz, CH_2CH=CH_2), 5.20 (dq, 1H, J = 10.3, 1.3 Hz, CH_2CH=CH_2), 4.79 (dt, 2H, J = 5.7, 1.3 Hz, CH_2CH=CH_2), 4.17 (br s, 2H, NH_2); ^{13}C NMR (151.0 MHz, DMSO) δ: 163.92 (s, C(O)), 163.75 (s, C(O)), 148.35, 134.05, 133.46, 132.03, 127.39, 125.96, 122.93, 122.26, 122.13, 121.11, 116.65, 112.35, 42.08 (s, CH_2CH=CH_2). Anal. Calcd for $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10; found: C, 71.14; H, 4.88; N, 10.96.

4.1.4. *N*-(2-Propenyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)acetamide (12d)

N-(2-Propenyl)-3-amino-1,8-naphthalimide **12c** (1.00 mmol) was treated with acetic anhydride (3.00 mL, 31.55 mmol) and stirred for 5 h at room temperature. The reaction mixture was cooled to room temperature and cold water (10 mL) was added. Yellow precipitate was filtered off, washed several times with water and dried to afford naphthalimide **12d**. Yield: 86%; yellow amorphous solid mp 253–254 °C; IR (KBr, cm^{-1}) ν_{max}: 3291, 3213, 1703, 1663, 1630, 1565, 1465, 1424, 1335, 1264, 1236, 1180, 880, 783, 745; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.95 (s, 1H), 8.50 (d, 1H, J = 7.1 Hz), 8.27 (d, 1H, J = 2.2 Hz), 8.18 (d, 1H, J = 8.3 Hz), 7.73 (dd, 1H, J = 8.3, 7.1 Hz), 7.69 (br s, 1H, N*H*), 5.98 (ddt, 1H, J = 17.1, 10.5, 5.5 Hz, CH_2CH=CH_2), 5.31 (d, 1H, J = 17.1 Hz, CH_2CH=CH_2), 5.21 (d, 1H, J = 10.5 Hz, CH_2CH=CH_2), 4.80 (d, 2H, J = 5.5 Hz, CH_2CH=CH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR (151.0 MHz, DMSO) δ: 169.50 (s, C(O)NH), 163.46 (s, C(O)), 163.24 (s, C(O)), 138.47, 134.11, 133.29, 132.49, 129.29, 127.86, 124.28, 124.15, 122.80, 122.03, 120.93, 116.83, 42.21 (s, CH_2CH=CH_2), 24.56 (s, CH_3C(O)). Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52; found: C, 69.14; H, 4.88; N, 9.76.

4.2. General procedure for the preparation of acrylamides 11a–f

To a solution of an appropriate 3-amino-1,8-naphthalimide **16a–f** (1.00 mmol) in dichloromethane (2 mL) triethylamine (1.10 mmol) was added. The mixture was cooled in an ice bath and acryloyl chloride (1.05 mmol) was added dropwise. The reaction mixture was stirred for 24 h at room temperature and extracted with water (3 × 3 mL). Subsequently, the inorganic layer was extracted with ethyl ether (3 × 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and filtered. After evaporation of solvents the residue was purified on a silica column with chloroform/methanol mixtures (100:1, 50:1 v/v) to afford the respective acrylamides **11a–f**.

4.2.1. *N*-(2-Propyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)propenamide (11a)

Yield: 57%; slightly yellowish amorphous solid (crystallized from chloroform/hexane) mp 223–224 °C; IR (KBr, cm^{-1}) ν_{max}: 3308, 2960, 1703, 1663, 1623, 1564, 1337, 1225, 786; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.05 (s, 1H), 8.49 (dd, 1H, J = 7.3, 1.0 Hz), 8.30 (d, 1H, J = 2.2 Hz), 8.17 (dd, 1H, J = 8.3, 1.0 Hz), 7.87 (br s, 1H, N*H*), 7.72 (dd, 1H, J = 8.3, 7.3 Hz), 6.55 (dd, 1H, J = 16.8, 1.2 Hz, CH=CH_2), 6.37 (dd, 1H, J = 16.8, 10.1 Hz, C*H*=CH_2), 5.89 (dd, 1H, J = 10.1, 1.2 Hz, CH=CH_2), 4.13 (t, 2H, J = 7.6 Hz, C$H_2CH_2CH_3$), 1.79–1.69 (m, 2H, $CH_2CH_2CH_3$), 1.01 (t, 3H, J = 7.4 Hz, $CH_2CH_2CH_3$); ^{13}C NMR (151.0 MHz, DMSO) δ: 164.24 (s, C(O)), 163.80 (s, C(O)), 163.58 (s, C(O)), 138.24, 134.12, 132.50, 131.97, 129.47, 128.22, 128.00, 124.54, 124.30, 123.12, 122.27, 121.48, 41.73 (s, $CH_2CH_2CH_3$), 21.31 (s, $CH_2CH_2CH_3$), 18.82 (s, $CH_2CH_2CH_3$). Anal. Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09; found: C, 70.37; H, 5.13; N, 8.98.

4.2.2. *N*-(2-Butyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)propenamide (11b)

Yield: 70%; slightly yellowish amorphous solid (crystallized from chloroform/hexane) mp 235–236 °C; IR (KBr, cm^{-1}) ν_{max}: 3258, 2956, 1696, 1661, 1624, 1547, 1338, 1269, 1227, 1130, 786; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.06 (d, 1H, J = 2.2 Hz), 8.49 (dd, 1H, J = 7.3, 1.0 Hz), 8.30 (d, 1H, J = 2.2 Hz), 8.17 (dd, 1H, J = 8.3, 1.0 Hz), 7.80 (br s, 1H, N*H*), 7.73 (dd, 1H, J = 8.3, 7.3 Hz), 6.55 (dd, 1H, J = 16.9, 1.2 Hz, CH=CH_2), 6.36 (dd, 1H, J = 16.9, 10.1 Hz, C*H*=CH_2), 5.89 (dd, 1H, J = 10.1, 1.2 Hz, CH=CH_2), 4.17 (t, 2H, J = 7.5 Hz, C$H_2CH_2CH_2CH_3$), 1.76–1.66 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.51–1.41 (m, 2H, $CH_2CH_2CH_2CH_3$), 0.97 (t, 3H, J = 7.1 Hz, $CH_2CH_2CH_2CH_3$); ^{13}C NMR (151.0 MHz, DMSO) δ: 164.24 (s, C(O)), 163.78 (s, C(O)), 163.55 (s, C(O)), 138.24, 134.13, 132.50, 131.97, 129.47, 128.23, 128.01, 124.54, 124.31, 123.15, 122.30, 121.50, 40.43 (s, N$CH_2CH_2CH_2CH_3$), 30.11 (s, $CH_2CH_2CH_2CH_3$), 20.28 (s, $CH_2CH_2CH_2CH_3$), 14.15 (s, $CH_2CH_2CH_2CH_3$). Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69; found: C, 70.44; H, 5.78; N, 8.43.

4.2.3. *N*-(2-Isobutyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)propenamide (11c)

Yield: 76%; slightly yellowish amorphous solid (crystallized from chloroform/hexane) mp 213–214 °C; IR (KBr, cm^{-1}) ν_{max}: 3316, 2959, 1702, 1661, 1621, 1562, 1467, 1418, 1371, 1337, 1264, 1227, 888, 787; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.06 (d, 1H, J = 1.8 Hz), 8.49 (dd, 1H, J = 7.2, 1.1 Hz), 8.31 (d, 1H, J = 1.8 Hz), 8.17 (dd, 1H, J = 8.1, 1.1 Hz), 7.89 (br s, 1H, N*H*), 7.72 (dd, 1H, J = 8.1, 7.2 Hz), 6.55 (dd, 1H, J = 16.9, 1.2 Hz, CH=CH_2), 6.38 (dd, 1H, J = 16.9, 10.1 Hz, C*H*=CH_2), 5.89 (dd, 1H, J = 10.1, 1.2 Hz, CH=CH_2), 4.04 (d, 2H, J = 7.3 Hz, C$H_2CH(CH_3)_2$), 2.29–2.16 (m, 1H, $CH_2CH(CH_3)_2$), 0.97 (d, 6H, J = 6.5 Hz, $CH_2CH(CH_3)_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 164.93 (s, C(O)), 164.66 (s, C(O)), 164.47 (s, C(O)), 137.36, 134.02, 132.51, 130.84, 130.03, 128.37, 127.46, 124.94, 124.45, 122.79, 122.71, 122.07, 47.43 (s, $CH_2CH(CH_3)_2$), 27.57 (s, $CH_2CH(CH_3)_2$), 20.42 (s, $CH_2CH(CH_3)_2$). Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69; found: C, 70.63; H, 5.84; N, 8.44.

4.2.4. *N*-(2-Dimethylamino)ethyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)propenamide (11d)

Yield: 30%; yellowish amorphous solid (crystallized from chloroform/hexane) mp 208–210 °C; IR (KBr, cm^{-1}) ν_{max}: 3275, 3096, 2774, 1660, 1623, 1561, 1467, 1415, 1267, 1231, 1138, 786; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.67 (br s, 1H, N*H*), 8.64 (s, 1H), 8.33 (dd, 1H, J = 7.3, 1.0 Hz), 7.99 (s, 1H), 7.90 (d, 1H, J = 8.2 Hz), 7.63 (dd, 1H, J = 8.2, 7.3 Hz), 6.52 (dd, 1H, J = 16.9, 1.6 Hz, CH=CH_2), 6.38 (dd, 1H, J = 16.9, 9.9 Hz, C*H*=CH_2), 5.86 (dd, 1H, J = 9.9, 1.6 Hz, CH=CH_2), 4.35 (t, 2H, J = 6.1 Hz, C$H_2CH_2N(CH_3)_2$), 2.86 (t, 2H, J = 6.1 Hz, $CH_2CH_2N(CH_3)_2$), 2.49 (s, 6H, $CH_2CH_2N(CH_3)_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 164.37 (s, C(O)), 163.98 (s, C(O)), 163.35 (s, C(O)), 136.76, 133.62, 131.85, 130.95, 129.50, 128.43, 127.28, 124.17, 123.55, 122.33, 121.67, 121.67, 57.85 (s, $CH_2CH_2N(CH_3)_2$), 46.05 (s, $CH_2CH_2N(CH_3)_2$), 37.89 (s, $CH_2CH_2N(CH_3)_2$). Anal. Calcd for $C_{19}H_{19}N_3O_3$: C, 67.64; H, 5.68; N, 12.46; found: C, 67.60; H, 5.43; N, 12.16.

4.2.5. *N*-(2-Diethylamino)ethyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)propenamide (11e)

Yield: 64%; yellowish amorphous solid (crystallized from chloroform/hexane) mp 196–197 °C; IR (KBr, cm^{-1}) ν_{max}: 3278, 2969, 1700, 1662, 1624, 1542, 1466, 1341, 1263, 1227, 784; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.96 (d, 1H, J = 2.1 Hz), 8.42 (dd, 1H, J = 7.2, 1.1 Hz), 8.41 (br s, 1H, N*H*), 8.30 (d, 1H, J = 2.1 Hz), 8.08 (dd, 1H, J = 8.2, 1.1 Hz), 7.67 (dd, 1H, J = 8.2, 7.2 Hz), 6.54 (dd, 1H, J = 16.9, 1.4 Hz, CH=CH_2), 6.40 (dd, 1H, J = 16.9, 10.0 Hz, C*H*=CH_2), 5.86 (dd, 1H, J = 10.0, 1.4 Hz, CH=CH_2), 4.27–4.22 (m, 2H, C$H_2CH_2N(CH_2CH_3)_2$), 2.78–2.73 (m, 2H, $CH_2CH_2N(CH_2CH_3)_2$), 2.64 (q, 4H, J = 7.1 Hz, $CH_2CH_2N(CH_2CH_3)_2$), 1.05 (t, 6H, J = 7.1 Hz, $CH_2CH_2N(CH_2CH_3)_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 164.49 (s, C(O)), 163.84 (s, C(O)), 163.77 (s, C(O)), 136.85, 133.81, 132.29, 130.83, 129.99, 128.90, 127.51, 124.84, 124.37, 122.88, 122.78, 122.00, 50.07 (s, $CH_2CH_2N(CH_2CH_3)_2$), 47.66 (s, $CH_2CH_2N(CH_2CH_3)_2$), 38.36 (s, $CH_2CH_2N(CH_2CH_3)_2$), 12.36 (s, $CH_2CH_2N(CH_2CH_3)_2$). Anal. Calcd for $C_{21}H_{23}N_3O_3$: C, 69.02; H, 6.34; N, 11.50; found: C, 68.88; H, 6.19; N, 11.34.

4.2.6. *N*-(2-Pirolidyn-1-yl)ethyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)propenamide (11f)

Yield: 43%; yellowish amorphous solid (crystallized from chloroform/hexane) mp 188–189 °C; IR (KBr, cm^{-1}) ν_{max}: 3254, 3096, 2966, 2786, 1698, 1661, 1562, 1467, 1418, 1349, 1268, 1232, 1131, 787; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.77 (d, 1H, J = 1.9 Hz), 8.37 (dd, 1H, J = 7.1, 1.0 Hz), 8.37 (br s, 1H, N*H*), 8.10 (d, 1H, J = 1.9 Hz), 7.99 (dd, 1H, J = 8.3, 1.0 Hz), 7.66 (dd, 1H, J = 8.3, 7.1 Hz), 6.53 (dd, 1H, J = 16.9, 1.6 Hz, CH=CH_2), 6.36 (dd, 1H, J = 16.9, 10.1 Hz, C*H*=CH_2), 5.87 (dd, 1H, J = 10.1, 1.6 Hz, CH=CH_2), 4.37 (t, 2H, J = 6.6 Hz, C$H_2CH_2N(CH_2CH_2)_2$), 2.94 (t, 2H, J = 6.6 Hz, $CH_2CH_2N(CH_2CH_2)_2$), 2.80–2.71 (m, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 1.86–1.79 (m, 4H, $CH_2CH_2N(CH_2CH_2)_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 164.25 (s, C(O)), 164.20 (s, C(O)), 163.60 (s, C(O)), 136.68, 133.83, 132.24, 130.87, 129.87, 128.84, 127.60, 124.70, 123.70, 122.95, 122.11, 121.95, 54.87 (s, $CH_2CH_2N(CH_2CH_2)_2$ and $CH_2CH_2N(CH_2CH_2)_2$), 39.38 (s, $CH_2CH_2N(CH_2CH_2)_2$), 23.91 (s, $CH_2CH_2N(CH_2CH_2)_2$). Anal. Calcd for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56; found: C, 69.53; H, 5.74; N, 11.67.

4.3. General procedure for the preparation of isoxazolidines *trans*-9a–f and *cis*-9a–f

A mixture of the nitrone **8** (1.00 mmol), acrylamide **11a–f** (1.00 mmol) and toluene or a toluene–chloroform mixture (2 mL, 1:1, v/v) was stirred at 70 °C for 24 h or until disappearance of the starting nitrone. After evaporation of solvents under reduced pressure the crude products were purified by silica gel chromatography with chloroform/methanol mixtures.

4.3.1. Diethyl *cis*-5-(*N*-propylnaphthalimide-3-ylcarbamoyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*cis*-9a)

Yellow oil; IR (film, cm^{-1}) ν_{max}: 3317, 2968, 2934, 1696, 1661, 1542, 1430, 1339, 1234, 1052, 1025, 972; ^1H NMR (300 MHz, $CDCl_3$) δ: 9.31 (br s, 1H, N*H*), 8.94 (d, 1H, J = 2.1 Hz), 8.50 (dd, 1H, J = 7.3, 1.0 Hz), 8.41 (d, 1H, J = 2.1 Hz), 8.18 (dd, 1H, J = 8.1, 1.0 Hz), 7.73 (dd, 1H, J = 8.1, 7.3 Hz), 4.70 (dd, 1H, J = 8.7, 5.0 Hz, *H*C5), 4.24–4.05 (m, 6H, 2 × CH_2OP and C$H_2CH_2CH_3$), 3.20–2.98 (m, 2H, *H*C3 and H_βC4), 3.02 (s, 3H, CH_3N), 2.94–2.81 (m, 1H, H_αC4), 1.80–1.70 (m, 2H, $CH_2CH_2CH_3$), 1.30 (t, 3H, J = 7.0 Hz, CH_3CH_2OP), 1.21 (t, 3H, J = 7.0 Hz, CH_3CH_2OP), 1.02 (t, 3H, J = 7.4 Hz, $CH_2CH_2CH_3$); ^{13}C NMR (151.0 MHz, $CDCl_3$) δ: 170.81 (s, C(O)NH), 164.11 (s, C(O)), 163.81 (s, C(O)), 136.32, 133.63, 132.55, 129.90, 127.49, 125.22, 124.20, 123.54, 122.55, 121.89, 76.02 (d, J = 6.8 Hz, C5), 63.72 (d, J = 169.4 Hz, C3), 63.07 (d, J = 7.1 Hz, CH_2OP), 62.02 (d, J = 6.8 Hz, CH_2OP), 46.04 (d, J = 5.8 Hz, CH_3N), 41.99 (s, $CH_2CH_2CH_3$), 36.41 (s, C4), 21.39 (s, $CH_2CH_2CH_3$), 16.43 (d, J = 6.0 Hz, CH_3CH_2OP), 16.36 (d, J = 5.6 Hz, CH_3CH_2OP), 11.49 (s, $CH_2CH_2CH_3$); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.31. Anal. Calcd for $C_{24}H_{30}N_3O_7P$: C, 57.25; H, 6.01; N, 8.35; found: C, 57.32; H, 5.92; N, 8.48.

4.3.2. Diethyl *trans*-5-(*N*-propylnaphtalimide-3-ylcarbamoyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-9a)

Yellowish amorphous solid (crystallized from chloroform/hexane) mp 175–176 °C; IR (KBr, cm^{-1}) ν_{max}: 3241, 3067, 2967, 1697, 1664, 1567, 1339, 1266, 1214, 1049, 1018; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.93 (d, 1H, J = 2.2 Hz), 8.57 (br s, 1H, N*H*), 8.51 (dd, 1H, J = 7.2, 1.1 Hz), 8.33 (d, 1H, J = 2.2 Hz), 8.18 (dd, 1H, J = 8.3, 1.1 Hz), 7.74 (dd, 1H, J = 8.3, 7.2 Hz), 4.69 (dd, 1H, J = 8.7, 5.8 Hz, *H*C5), 4.29–4.14 (m, 4H, 2 × C*H*$_2$OP), 4.15 (t, 2H, J = 7.5 Hz, C*H*$_2$CH$_2$CH$_3$), 3.19–3.06 (m, 1H, *H*C3), 3.08 (s, 3H, C*H*$_3$N), 3.07 (dddd, 1H, J = 16.1, 12.8, 8.7, 8.7 Hz, H_βC4), 2.88 (dddd, 1H, J = 12.8, 9.9, 8.2, 5.8 Hz, H_αC4), 1.80–1.72 (m, 2H, CH$_2$C*H*$_2$CH$_3$), 1.39 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP), 1.37 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP), 1.02 (t, 3H, J = 7.4 Hz, CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.45 (s, C(O)NH), 163.72 (s, C(O)), 163.37 (s, C(O)), 135.71, 133.47, 132.18, 129.89, 127.46, 125.00, 124.00, 123.25, 122.23, 122.12, 76.58 (d, J = 8.6 Hz, C5), 63.57 (d, J = 164.3 Hz, C3), 63.41 (d, J = 6.3 Hz, CH$_2$OP), 62.84 (d, J = 6.9 Hz, CH$_2$OP), 46.84 (s, CH$_3$N), 42.03 (s, *C*H$_2$CH$_2$CH$_3$), 36.52 (s, C4), 21.48 (s, CH$_2$*C*H$_2$CH$_3$), 16.71 (d, J = 5.1 Hz, *C*H$_3$CH$_2$OP), 16.61 (d, J = 5.1 Hz, *C*H$_3$CH$_2$OP), 11.67 (s, CH$_2$CH$_2$*C*H$_3$); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 20.52. Anal. Calcd for $C_{24}H_{30}N_3O_7P$: C, 57.25; H, 6.01; N, 8.35; found: C, 57.34; H, 5.92; N, 8.58.

4.3.3. Diethyl *cis*-5-(*N*-butylnaphthalimide-3-ylcarbamoyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*cis*-9b)

Yellow oil; IR (film, cm^{-1}) ν_{max}: 2962, 2932, 1697, 1662, 1543, 1466, 1430, 1340, 1233, 1053, 1026, 753; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.30 (br s, 1H, N*H*), 8.94 (d, 1H, J = 2.1 Hz), 8.50 (dd, 1H, J = 7.3, 1.2 Hz), 8.40 (d, 1H, J = 2.1 Hz), 8.17 (dd, 1H, J = 8.3, 1.2 Hz), 7.72 (dd, 1H, J = 8.3, 7.3 Hz), 4.71–4.67 (m, 1H, *H*C5), 4.24–4.04 (m, 4H, 2 × C*H*$_2$OP), 4.16 (t, 2H, J = 7.4 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 3.14–3.05 (m, 2H, *H*C3 and H_βC4), 3.01 (d, 3H, J = 0.6 Hz, C*H*$_3$N), 2.87–2.78 (m, 1H, H_αC4), 1.77–1.67 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.51–1.39 (m, 2H, CH$_2$CH$_2$C*H*$_2$CH$_3$), 1.30 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP), 1.21 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP), 0.98 (t, 3H, J = 7.3 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (151.0 MHz, $CDCl_3$) δ: 170.80 (s, C(O)NH), 164.09 (s, C(O)), 163.78 (s, C(O)), 136.31, 133.62, 132.53, 129.87, 127.48, 125.19, 124.18, 123.52, 122.54, 121.87, 76.01 (d, J = 6.8 Hz, C5), 63.70 (d, J = 169.6 Hz, C3), 63.07 (d, J = 6.3 Hz, CH$_2$OP), 62.03 (d, J = 6.4 Hz, CH$_2$OP), 46.03 (d, J = 5.8 Hz, CH$_3$N), 40.28 (s, *C*H$_2$CH$_2$N(CH$_3$)$_2$), 36.39 (s, C4), 30.21 (s, CH$_2$CH$_2$CH$_2$CH$_3$), 20.35 (s, CH$_2$CH$_2$CH$_2$CH$_3$), 16.43 (d, J = 5.8 Hz, *C*H$_3$CH$_2$OP), 16.35 (d, J = 5.9 Hz, *C*H$_3$CH$_2$OP), 13.81 (s, CH$_2$CH$_2$CH$_2$*C*H$_3$); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.37. Anal. Calcd for $C_{25}H_{32}N_3O_7P$: C, 58.02; H, 6.23; N, 8.12; found: C, 58.30; H, 6.23; N, 8.00.

4.3.4. Diethyl *trans*-5-(*N*-butylnaphthalimide-3-ylcarbamoyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-9b)

Yellowish amorphous solid (crystallized from chloroform/hexane) mp 157–158 °C; IR (KBr, cm^{-1}) ν_{max}: 3278, 2958, 1698, 1661, 1565, 1341, 1229, 1052, 1033, 970; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.93 (d, 1H, J = 2.2 Hz), 8.57 (br s, 1H, N*H*), 8.51 (dd, 1H, J = 7.3, 1.2 Hz), 8.33 (d, 1H, J = 2.2 Hz), 8.18 (dd, 1H, J = 8.3, 1.2 Hz), 7.74 (dd, 1H, J = 8.3, 7.3 Hz), 4.69 (dd, 1H, J = 8.5, 5.6 Hz, *H*C5), 4.29–4.16 (m, 4H, 2 × C*H*$_2$OP), 4.18 (t, 2H, J = 7.4 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 3.18–3.05 (m, 1H, *H*C3), 3.07 (s, 3H, C*H*$_3$N), 3.07 (dddd, 1H, J = 15.9, 12.7, 8.5, 8.5 Hz, H_βC4), 2.88 (dddd, 1H, J = 12.7, 10.1, 8.1, 5.6 Hz, H_αC4), 1.76–1.66 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.51–1.39 (m, 2H, CH$_2$CH$_2$C*H*$_2$CH$_3$), 1.38 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP), 1.37 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP), 0.98 (t, 3H, J = 7.3 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.47 (s, C(O)NH), 163.79 (s, C(O)), 163.45 (s, C(O)), 135.70, 133.55, 132.26, 129.98, 127.54, 125.10, 124.01, 123.36, 122.33, 122.17, 76.59 (d, J = 8.9 Hz, C5), 63.59 (d, J = 167.2 Hz, C3), 63.47 (d, J = 6.6 Hz, CH$_2$OP), 62.87 (d, J = 6.9 Hz, CH$_2$OP), 46.86 (s, CH$_3$N), 40.37 (s, *C*H$_2$CH$_2$CH$_2$CH$_3$), 36.56 (s, C4), 30.31 (s, CH$_2$CH$_2$CH$_2$CH$_3$), 20.51 (s, CH$_2$CH$_2$CH$_2$CH$_3$), 16.74 (d, J = 5.1 Hz, *C*H$_3$CH$_2$OP), 16.64 (d, J = 5.1 Hz, *C*H$_3$CH$_2$OP), 14.02 (s, CH$_2$CH$_2$CH$_2$*C*H$_3$); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 20.55. Anal. Calcd for $C_{25}H_{32}N_3O_7P$: C, 58.02; H, 6.23; N, 8.12; found: C, 58.13; H, 6.20; N, 7.92.

4.3.5. Diethyl *cis*-5-(*N*-isobutylnaphthalimide-3-ylcarbamoyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*cis*-9c)

Yellow oil; IR (film, cm^{-1}) ν_{max}: 2963, 1701, 1662, 1543, 1337, 1234, 1054, 1027, 755; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.30 (br s, 1H, N*H*), 8.95 (d, 1H, J = 2.2 Hz), 8.50 (dd, 1H, J = 7.3, 1.1 Hz), 8.40 (d, 1H, J = 2.2 Hz), 8.18 (dd, 1H, J = 8.3, 1.1 Hz), 7.73 (dd, 1H, J = 8.3, 7.3 Hz), 4.69 (dd, 1H, J = 8.3, 4.6 Hz, *H*C5), 4.24–4.02 (m, 4H, 2 × C*H*$_2$OP), 4.05 (d, 2H, J = 7.3 Hz, C*H*$_2$CH(CH$_3$)$_2$), 3.16–3.01 (m, 2H, *H*C3 and H_βC4), 3.01 (d, 3H, J = 1.0 Hz, C*H*$_3$N), 2.93–2.83 (m, 1H, H_αC4), 2.28–2.19 (m, 1H, CH$_2$C*H*(CH$_3$)$_2$), 1.30 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP), 1.21 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP), 0.99 (d, 6H, J = 6.7 Hz, CH$_2$CH(C*H*$_3$)$_2$); ^{13}C NMR (151.0 MHz, $CDCl_3$) δ: 170.80 (s, C(O)NH), 164.37 (s, C(O)), 164.08 (s, C(O)), 136.33, 133.59, 132.52, 129.95, 127.48, 125.23, 124.29, 123.46, 122.48, 121.86, 76.01 (d, J = 6.8 Hz, C5), 63.70 (d, J = 166.5 Hz, C3), 63.06 (d, J = 6.5 Hz, CH$_2$OP), 63.02 (d, J = 6.8 Hz, CH$_2$OP), 47.21 (s, *C*H$_2$CH(CH$_3$)$_2$), 46.01 (d, J = 5.7 Hz, CH$_3$N), 36.39 (s, C4), 27.39 (s, CH$_2$*C*H(CH$_3$)$_2$), 20.28 (s, CH$_2$CH(*C*H$_3$)$_2$), 16.42 (d, J = 5.6 Hz, *C*H$_3$CH$_2$OP), 16.34 (d, J = 5.6 Hz, *C*H$_3$CH$_2$OP); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.36. Anal. Calcd for $C_{25}H_{32}N_3O_7P$: C, 58.02; H, 6.23; N, 8.12; found: C, 58.15; H, 6.25; N, 7.87.

4.3.6. Diethyl *trans*-5-(*N*-isobutylnaphthalimide-3-ylcarbamoyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-9c)

Yellowish amorphous solid (crystallized from chloroform/hexane) mp 165–166 °C; IR (KBr, cm^{-1}) ν_{max}: 3273, 3243, 2986, 2955, 1699, 1661, 1564, 1337, 1228, 1052, 1034, 973, 783; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.93 (d, 1H, J = 2.2 Hz), 8.57 (br s, 1H, N*H*), 8.51 (dd, 1H, J = 7.3, 1.0 Hz), 8.33 (d, 1H, J = 2.2 Hz), 8.17 (dd, 1H, J = 8.3, 1.2 Hz), 7.74 (dd, 1H, J = 8.3, 7.3 Hz), 4.69 (dd, 1H, J = 8.7, 5.7 Hz, *H*C5), 4.29–4.18 (m, 4H, 2 × C*H*$_2$OP), 4.04 (d, 2H, J = 7.5 Hz, C*H*$_2$CH(CH$_3$)$_2$), 3.16–3.05 (m, 1H), 3.07 (s, 3H, C*H*$_3$N), 3.06 (dddd, 1H, J = 15.7, 12.8, 8.7, 8.7 Hz, H_βC4), 2.87 (dddd, 1H, J = 12.8, 10.3, 8.1, 5.7 Hz, H_αC4), 2.28–2.19 (m, 1H, CH$_2$C*H*(CH$_3$)$_2$), 1.38 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP), 1.37 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP), 0.98 (d, 6H, J = 6.5 Hz, CH$_2$CH(C*H*$_3$)$_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.47 (s, C(O)NH), 164.21 (s, C(O)), 163.90 (s, C(O)), 135.69, 133.65, 132.43, 130.23, 127.68, 125.34, 124.13, 123.53, 122.46, 122.26, 76.53 (d, J = 8.9 Hz, C5), 63.68 (d, J = 166.9 Hz, C3), 63.54 (d, J = 6.6 Hz, CH$_2$OP), 62.89 (d, J = 6.9 Hz, CH$_2$OP), 47.38 (s, *C*H$_2$CH(CH$_3$)$_2$), 46.93 (br s, CH$_3$N), 36.72 (s, C4), 27.60 (s, CH$_2$*C*H(CH$_3$)$_2$), 20.52 (s, CH$_2$CH(*C*H$_3$)$_2$), 16.78 (d, J = 5.1 Hz, *C*H$_3$CH$_2$OP), 16.71 (d, J = 5.4 Hz, *C*H$_3$CH$_2$OP); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 20.53. Anal. Calcd for $C_{25}H_{32}N_3O_7P$: C, 58.02; H, 6.23; N, 8.12; found: C, 58.12; H, 5.99; N, 7.88.

4.3.7. Diethyl *cis*-5-[*N*-(2-dimethylamino)ethylnaphthalimide-3-ylcarbamoyl]-2-methylisoxazolidin-3-yl-3-phosphonate (*cis*-9d)

Yellow amorphous solid; mp 119–120 °C; IR (KBr, cm^{-1}) ν_{max}: 3274, 2980, 2776, 1695, 1662, 1544, 1465, 1430, 1341, 1237, 1026, 971, 751; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.29 (br s, 1H, N*H*), 8.95 (d, 1H, J = 2.2 Hz), 8.50 (dd, 1H, J = 7.4, 1.2 Hz), 8.41 (d, 1H, J = 2.2 Hz), 8.18 (d, 1H, J = 8.3 Hz), 7.72 (dd, 1H, J = 8.3, 7.4 Hz), 4.69 (dd, 1H, J = 8.9, 4.8 Hz, *H*C5), 4.34 (t, 2H, J = 6.9 Hz, C*H*$_2$CH$_2$N(CH$_3$)$_2$), 4.23–4.05 (m, 4H, 2 × C*H*$_2$OP), 3.18–

3.00 (m, 2H, *H*C3 and H_βC4), 3.01 (s, 3H, CH_3N), 2.87–2.78 (m, 1H, H_αC4), 2.69 (t, 2H, J = 6.9 Hz, $CH_2CH_2N(CH_3)_2$), 2.38 (s, 6H, $CH_2CH_2N(CH_3)_2$), 1.30 (t, 3H, J = 7.0 Hz, CH_3CH_2OP), 1.20 (t, 3H, J = 7.0 Hz, CH_3CH_2OP); ^{13}C NMR (151.0 MHz, $CDCl_3$) δ: 170.83 (s, C(O)NH), 164.13 (s, C(O)), 163.83 (s, C(O)), 136.33, 133.73, 132.56, 129.98, 127.49, 125.26, 124.25, 123.45, 122.46, 121.93, 76.00 (d, J = 6.8 Hz, C5), 63.71 (d, J = 169.5 Hz, C3), 63.07 (d, J = 6.7 Hz, CH_2OP), 63.02 (d, J = 7.0 Hz, CH_2OP), 56.95 (s, $CH_2CH_2N(CH_3)_2$), 46.03 (d, J = 5.9 Hz, CH_3N), 45.67 (s, $CH_2CH_2N(CH_3)_2$), 38.15 (s, $CH_2CH_2N(CH_3)_2$), 36.41 (s, C4), 16.43 (d, J = 5.6 Hz, CH_3CH_2OP), 16.36 (d, J = 5.9 Hz, CH_3CH_2OP); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.32. Anal. Calcd for $C_{25}H_{33}N_4O_7P$: C, 56.39; H, 6.25; N, 10.52; found: C, 56.20; H, 6.49; N, 10.39.

4.3.8. Diethyl *trans*-5-[*N*-(2-dimethylamino)ethylnaphthalimide-3-ylcarbamoyl]-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-9d)

Yellowish oil; IR (film, cm^{-1}) ν_{max}: 3248, 3092, 2943, 2776, 1700, 1662, 1562, 1465, 1430, 1340, 1236, 1052, 1025, 970, 751; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.91 (d, 1H, J = 2.1 Hz), 8.61 (br s, 1H, N*H*), 8.50 (d, 1H, J = 7.1 Hz), 8.32 (d, 1H, J = 2.1 Hz), 8.16 (d, 1H, J = 8.5 Hz), 7.73 (dd, 1H, J = 8.5, 7.1 Hz), 4.69 (dd, 1H, J = 8.7, 5.8 Hz, *H*C5), 4.33 (t, 2H, J = 6.8 Hz, $CH_2CH_2N(CH_3)_2$), 4.29–4.16 (m, 4H, 2 × CH_2OP), 3.18–3.05 (m, 1H, *H*C3), 3.07 (s, 3H, CH_3N), 3.06 (dddd, 1H, J = 16.0, 12.8, 8.7, 8.7 Hz, H_βC4), 2.88 (dddd, 1H, J = 12.8, 9.6, 8.2, 5.8 Hz, H_αC4), 2.66 (t, 2H, J = 6.8 Hz, $CH_2CH_2N(CH_3)_2$), 2.36 (s, 6H, $CH_2CH_2N(CH_3)_2$), 1.38 (t, 3H, J = 7.1 Hz, CH_3CH_2OP), 1.37 (t, 3H, J = 7.0 Hz, CH_3CH_2OP); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.42 (s, C(O)NH), 163.92 (s, C(O)), 163.53 (s, C(O)), 135.71, 133.69, 132.30, 130.08, 127.58, 125.13, 123.93, 123.31, 122.28, 122.09, 76.57 (d, J = 8.9 Hz, C5), 63.65 (d, J = 170.9 Hz, C3), 63.50 (d, J = 6.6 Hz, CH_2OP), 62.86 (d, J = 6.9 Hz, CH_2OP), 57.21 (s, $CH_2CH_2N(CH_3)_2$), 46.95 (s, CH_3N), 45.90 (s, $CH_2CH_2N(CH_3)_2$), 38.33 (s, $CH_2CH_2N(CH_3)_2$), 36.57 (s, C4), 16.79 (d, J = 5.1 Hz, CH_3CH_2OP), 16.68 (d, J = 5.2 Hz, CH_3CH_2OP); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 20.56. Anal. Calcd for $C_{25}H_{33}N_4O_7P$: C, 56.39; H, 6.25; N, 10.52; found: C, 56.18; H, 6.48; N, 10.45.

4.3.9. Diethyl *cis*-5-[*N*-(2-diethylamino)ethylnaphthalimide-3-ylcarbamoyl]-2-methylisoxazolidin-3-yl-3-phosphonate (*cis*-9e)

Yellowish oil; IR (film, cm^{-1}) ν_{max}: 3301, 2973, 2932, 1696, 1662, 1544, 1466, 1430, 1342, 1235, 1026, 972, 785; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.29 (br s, 1H, N*H*), 8.95 (d, 1H, J = 2.2 Hz), 8.49 (dd, 1H, J = 7.3, 1.2 Hz), 8.40 (d, 1H, J = 2.2 Hz), 8.18 (d, 1H, J = 8.3 Hz), 7.72 (dd, 1H, J = 8.3, 7.3 Hz), 4.69 (dd, 1H, J = 8.7, 4.7 Hz, *H*C5), 4.31 (t, 2H, J = 7.1 Hz, $CH_2CH_2N(CH_2CH_3)_2$), 4.23–4.04 (m, 4H, 2 × CH_2OP), 3.20–2.98 (m, 2H, *H*C3 and H_βC4), 3.01 (s, 3H, CH_3N), 2.98–2.80 (m, 1H, H_αC4), 2.83 (t, 2H, J = 7.1 Hz, $CH_2CH_2N(CH_2CH_3)_2$), 2.69 (q, 4H, J = 7.0 Hz, $CH_2CH_2N(CH_2CH_3)_2$), 1.29 (t, 3H, J = 7.0 Hz, CH_3CH_2OP), 1.20 (t, 3H, J = 7.0 Hz, CH_3CH_2OP), 1.11 (t, 6H, J = 7.0 Hz, $CH_2CH_2N(CH_2CH_3)_2$); ^{13}C NMR (151.0 MHz, $CDCl_3$) δ: 170.86 (s, C(O)NH), 164.08 (s, C(O)), 163.78 (s, C(O)), 136.34, 133.77, 132.60, 129.95, 127.53, 125.23, 124.20, 123.44, 122.45, 121.98, 76.01 (d, J = 6.8 Hz, C5), 63.71 (d, J = 169.4 Hz, C3), 63.08 (d, J = 6.7 Hz, CH_2OP), 63.01 (d, J = 6.8 Hz, CH_2OP), 49.69 (s, $CH_2CH_2N(CH_2CH_3)_2$), 47.67 (s, $CH_2CH_2N(CH_2CH_3)_2$), 46.04 (d, J = 5.8 Hz, CH_3N), 37.85 (s, $CH_2CH_2N(CH_2CH_3)_2$), 36.45 (s, C4), 16.43 (d, J = 5.6 Hz, CH_3CH_2OP), 16.36 (d, J = 6.0 Hz, CH_3CH_2OP), 12.06 (s, $CH_2CH_2N(CH_2CH_3)_2$); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.31. Anal. Calcd for $C_{27}H_{37}N_4O_7P$: C, 57.85; H, 6.65; N, 9.99; found: C, 57.67; H, 6.62; N, 9.85.

4.3.10. Diethyl *trans*-5-[*N*-(2-diethylamino)ethylnaphthalimide-3-ylcarbamoyl]-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-9e)

Yellowish oil; IR (film, cm^{-1}) ν_{max}: 3249, 2974, 2933, 1698, 1662, 1563, 1466, 1430, 1341, 1235, 1054, 1027, 972, 755; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.93 (d, 1H, J = 2.2 Hz), 8.64 (br s, 1H, N*H*), 8.51 (dd, 1H, J = 7.3, 0.8 Hz), 8.35 (d, 1H, J = 2.2 Hz), 8.18 (d, 1H, J = 8.0 Hz), 7.74 (dd, 1H, J = 8.0, 7.3 Hz), 4.71 (dd, 1H, J = 8.8, 5.6 Hz, *H*C5), 4.30 (t, 2H, J = 7.5 Hz, $CH_2CH_2N(CH_2CH_3)_2$), 4.28–4.19 (m, 4H, 2 × CH_2OP), 3.18–3.11 (m, 1H, *H*C3), 3.09 (s, 3H, CH_3N), 3.08 (dddd, 1H, J = 16.0, 12.7, 8.8, 8.8 Hz, H_βC4), 2.89 (dddd, 1H, J = 12.7, 9.5, 8.3, 5.6 Hz, H_αC4), 2.80 (t, 2H, J = 7.5 Hz, $CH_2CH_2N(CH_2CH_3)_2$), 2.68 (q, 4H, J = 7.1 Hz, $CH_2CH_2N(CH_2CH_3)_2$), 1.40 (t, 3H, J = 7.0 Hz, CH_3CH_2OP), 1.38 (t, 3H, J = 7.0 Hz, CH_3CH_2OP), 1.10 (t, 6H, J = 7.1 Hz, $CH_2CH_2N(CH_2CH_3)_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.46 (s, C(O)NH), 163.81 (s, C(O)), 163.47 (s, C(O)), 135.71, 133.68, 132.34, 130.04, 127.59, 125.16, 124.01, 123.35, 122.32, 122.23, 76.57 (d, J = 7.7 Hz, C5), 63.64 (d, J = 167.8 Hz, C3), 63.48 (d, J = 6.6 Hz, CH_2OP), 62.86 (d, J = 6.9 Hz, CH_2OP), 49.87 (s, $CH_2CH_2N(CH_2CH_3)_2$), 47.72 (s, $CH_2CH_2N(CH_2CH_3)_2$), 46.96 (s, CH_3N), 38.16 (s, $CH_2CH_2N(CH_2CH_3)_2$), 36.63 (s, C4), 16.76 (d, J = 4.9 Hz, CH_3CH_2OP), 16.65 (d, J = 5.4 Hz, CH_3CH_2OP), 12.36 (s, $CH_2CH_2N(CH_2CH_3)_2$); ^{31}P NMR (243.0 MHz, $CDCl_3$) δ: 20.17. Anal. Calcd for $C_{27}H_{37}N_4O_7P$: C, 57.85; H, 6.65; N, 9.99; found: C, 57.87; H, 6.71; N, 9.91.

4.3.11. Diethyl *cis*-5-[*N*-(2-pirolidyn-1-yl)ethylnaphthalimide-3-ylcarbamoyl]-2-methylisoxazolidin-3-yl-3-phosphonate (*cis*-9f)

Yellowish oil; IR (film, cm^{-1}) ν_{max}: 3276, 2971, 2791, 1691, 1662, 1543, 1430, 1338, 1234, 1052, 971, 785, 750; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.30 (br s, 1H, N*H*), 8.96 (d, 1H, J = 2.0 Hz), 8.52 (d, 1H, J = 7.2 Hz), 8.43 (d, 1H, J = 2.0 Hz), 8.19 (d, 1H, J = 8.1 Hz), 7.74 (dd, 1H, J = 8.1, 7.2 Hz), 4.71 (dd, 1H, J = 9.2, 5.0 Hz, *H*C5), 4.40 (t, 2H, J = 7.3 Hz, $CH_2CH_2N(CH_2CH_2)_2$), 4.24–4.08 (m, 4H, 2 × CH_2OP), 3.15–3.08 (m, 2H, *H*C3 and H_βC4), 3.04 (s, 3H, CH_3N), 2.93–2.88 (m, 1H, H_αC4), 2.88–2.83 (m, 2H, $CH_2CH_2N(CH_2CH_2)_2$), 2.71 (br s, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 1.83 (br s, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 1.32 (t, 3H, J = 7.0 Hz, CH_3CH_2OP), 1.23 (t, 3H, J = 7.0 Hz, CH_3CH_2OP); ^{13}C NMR (151.0 MHz, $CDCl_3$) δ: 170.84 (s, C(O)NH), 164.07 (s, C(O)), 163.75 (s, C(O)), 136.32, 133.72, 132.58, 129.95, 127.50, 125.26, 124.21, 123.50, 122.51, 121.92, 76.00 (d, J = 7.3 Hz, C5), 63.72 (d, J = 169.1 Hz, C3), 63.08 (d, J = 6.7 Hz, CH_2OP), 62.99 (d, J = 6.8 Hz, CH_2OP), 54.35 (s, $CH_2CH_2N(CH_2CH_2)_2$), 53.63 (s, $CH_2CH_2N(CH_2CH_2)_2$), 46.04 (d, J = 5.7 Hz, CH_3N), 39.21 (s, $CH_2CH_2N(CH_2CH_3)_2$), 36.44 (s, C4), 23.64 (s, $CH_2CH_2N(CH_2CH_2)_2$, 16.43 (d, J = 5.7 Hz, CH_3CH_2OP), 16.36 (d, J = 5.6 Hz, CH_3CH_2OP); ^{31}P NMR (243 MHz, $CDCl_3$) δ: 20.86. Anal. Calcd for $C_{27}H_{35}N_4O_7P$: C, 58.06; H, 6.32; N, 10.03; found: C, 57.84; H, 6.39; N, 9.75.

4.3.12. Diethyl *trans*-5-[*N*-(2-pirolidyn-1-yl)ethylnaphthalimide-3-ylcarbamoyl]-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-9f)

Yellowish oil; IR (film, cm^{-1}) ν_{max}: 3271, 2970, 1698, 1661, 1563, 1465, 1430, 1337, 1233, 1027, 970, 784, 749; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.94 (d, 1H, J = 2.1 Hz), 8.60 (br s, 1H, N*H*), 8.53 (dd, 1H, J = 7.3, 0.9 Hz), 8.36 (d, 1H, J = 2.1 Hz), 8.19 (d, 1H, J = 8.0 Hz), 7.76 (dd, 1H, J = 8.0, 7.3 Hz), 4.71 (dd, 1H, J = 8.9, 5.5 Hz, *H*C5), 4.39 (t, 2H, J = 7.3 Hz, $CH_2CH_2N(CH_2CH_2)_2$), 4.29–4.19 (m, 4H, 2 × CH_2OP), 3.17–3.09 (m, 1H, *H*C3), 3.10 (s, 3H, CH_3N), 3.08 (dddd, 1H, J = 16.1, 12.9, 8.9, 8.9 Hz, H_βC4), 2.89 (dddd, 1H, J = 12.9, 9.4, 8.3, 5.5 Hz, H_αC4), 2.85 (t, 2H, J = 7.3 Hz, $CH_2CH_2N(CH_2CH_2)_2$), 2.70 (br s, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 1.82 (br s, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 1.41 (t, 3H, J = 7.1 Hz, CH_3CH_2OP),

1.39 (t, 3H, J = 7.1 Hz, CH_3CH$_2$OP); ^{13}C NMR (151.0 MHz, $CDCl_3$) δ: 169.48 (s, C(O)NH), 163.88 (s, C(O)), 163.55 (s, C(O)), 135.68, 133.64, 132.40, 130.09, 127.59, 125.27, 123.97, 123.49, 122.43, 122.20, 76.38 (d, J = 8.9 Hz, C5), 63.67 (d, J = 170.4 Hz, C3), 63.35 (d, J = 6.5 Hz, CH$_2$OP), 62.69 (d, J = 6.9 Hz, CH$_2$OP), 54.34 (s, CH$_2$CH$_2$N(CH$_2$CH$_2$)$_2$), 53.64 (s, CH$_2$CH$_2$N(CH$_2$CH$_2$)$_2$), 46.72 (s, CH$_3$N), 39.27 (s, CH$_2$CH$_2$N(CH$_2$CH$_3$)$_2$), 36.56 (s, C4), 23.61 (s, CH$_2$CH$_2$N(CH$_2$CH$_2$)$_2$, 16.52 (d, J = 5.9 Hz, CH$_3$CH$_2$OP), 16.45 (d, J = 5.7 Hz, CH$_3$CH$_2$OP); ^{31}P NMR (243.0 MHz, $CDCl_3$) δ: 20.13. Anal. Calcd for $C_{27}H_{35}N_4O_7P$: C, 58.06; H, 6.32; N, 10.03; found: C, 58.22; H, 6.28; N, 9.83.

4.4. General procedure for the preparation of isoxazolidines *trans*-10a–d and *cis*-10a–d

A mixture of the nitrone **8** (1.00 mmol), *N*-(2-propenyl)naphthalimide **12a–d** (1.00 mmol) and toluene or a toluene–chloroform mixture (2 mL, 1:1, v/v) was stirred at 70 °C for 24 h or until disappearance of the starting nitrone. After evaporation of solvents under reduced pressure the crude products were purified by silica gel chromatography with chloroform/methanol mixtures.

4.4.1. Diethyl *trans*-5-(1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]izochinolin-2-ylmethyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-10a)

White amorphous solid (crystallized from chloroform/hexane) mp 175–176 °C; IR (KBr, cm^{-1}) ν_{max}: 2981, 1702, 1662, 1591, 1328, 1234, 1056, 1035, 973; (signals of *trans*-**10a** were extracted from the spectra of a 93:7 mixture of *trans*-**10a** and *cis*-**10a**); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.61 (dd, 2H, J = 7.2, 1.1 Hz), 8.23 (dd, 2H, J = 8.1, 1.1 Hz), 7.76 (dd, 2H, J = 8.1, 7.2 Hz), 4.56–4.44 (m, 2H, *H*C5 and CH_2N), 4.33–4.28 (m, 1H, CH_2N), 4.22–4.11 (m, 4H, 2 × CH_2OP), 3.09 (ddd, 1H, J = 9.5, 7.2, 2.3 Hz, *H*C3), 2.89 (d, 3H, J = 0.8 Hz, CH_3N), 2.65 (dddd, 1H, J = 18.0, 12.5, 7.2, 7.2 Hz, H_βC4), 2.44 (dddd, 1H, J = 12.5, 11.6, 9.5, 6.9 Hz, H_αC4), 1.33 (t, 3H, J = 7.0 Hz, CH_3CH$_2$OP), 1.32 (t, 3H, J = 7.0 Hz, CH_3CH$_2$OP); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 164.16 (s, 2 × C(O)), 134.14, 131.60, 131.47, 128.21, 126.98, 122.50, 74.96 (d, J = 7.6 Hz, C5), 64.06 (d, J = 168.6 Hz, C3), 63.34 (d, J = 6.6 Hz, CH$_2$OP), 62.53 (d, J = 6.9 Hz, CH$_2$OP), 46.71 (br s, CH$_3$N), 42.77 (s, CH$_2$N), 36.55 (s, C4), 16.81 (d, J = 6.0 Hz, CH$_3$CH$_2$OP), 16.73 (d, J = 6.0 Hz, CH$_3$CH$_2$OP); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 22.67. Anal. Calcd for $C_{21}H_{25}N_2O_6P$: C, 58.33; H, 5.83; N, 6.48; found: C, 58.28; H, 5.59; N, 6.61 (obtained on a 93:7 mixture of *trans*-**10a** and *cis*-**10a**).

4.4.2. Diethyl *trans*-5-(5-nitro-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]izochinolin-2-ylmethyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-10b)

Grey amorphous solid (crystallized from chloroform/hexane) mp 197–198 °C; IR (KBr, cm^{-1}) ν_{max}: 2985, 1715, 1672, 1600, 1541, 1343, 1326, 1233, 1033, 974, 799; (signals of *trans*-**10b** were extracted from the spectra of a 94:6 mixture of *trans*-**10b** and *cis*-**10b**); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.32 (d, 1H, J = 2.2 Hz), 9.14 (d, 1H, J = 2.2 Hz), 8.79 (dd, 1H, J = 7.3, 1.2 Hz), 8.44 (dd, 1H, J = 8.2, 1.2 Hz), 7.95 (dd, 1H, J = 8.2, 7.3 Hz), 4.56 (dd, 1H, J = 12.5, 7.7 Hz, CH_2N), 4.47 (dddd, 1H, J = 7.7, 7.2, 7.2, 4.0 Hz, *H*C5), 4.30 (dd, 1H, J = 12.5, 4.0 Hz, CH_2N), 4.25–4.12 (m, 4H, 2 × CH_2OP), 3.09 (ddd, 1H, J = 9.6, 7.2, 2.4 Hz, *H*C3), 2.88 (d, 3H, J = 1.0 Hz, CH_3N), 2.69 (dddd, 1H, J = 18.1, 12.5, 7.2, 7.2 Hz, H_αC4), 2.41 (dddd, 1H, J = 12.5, 11.9, 9.6, 7.2 Hz, H_βC4), 1.34 (t, 3H, J = 7.1 Hz, CH_3CH$_2$OP), 1.33 (t, 3H, J = 7.0 Hz, CH_3CH$_2$OP); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 163.07 (s, C(O)), 162.50 (s, C(O)), 146.43, 135.66, 134.61, 131.04, 130.24, 129.09, 129.02, 124.55, 124.44, 123.07, 74.53 (d, J = 7.6 Hz, C5), 63.94 (d, J = 169.3 Hz, C3), 63.13 (d, J = 6.5 Hz, CH$_2$OP), 62.42 (d, J = 6.7 Hz, CH$_2$OP), 46.46 (s, CH$_3$N), 43.05 (s, CH$_2$N), 36.34 (s, C4), 16.51 (d, J = 6.3 Hz, CH$_3$CH$_2$OP), 16.47 (d, J = 6.1 Hz, CH$_3$CH$_2$OP); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 22.49. Anal. Calcd for $C_{21}H_{24}N_3O_8P$: C, 52.83; H, 5.07; N, 8.80; found: C, 52.73; H, 4.82; N, 8.52 (obtained on a 94:6 mixture of *trans*-**10b** and *cis*-**10b**).

4.4.3. Diethyl *trans*-5-(5-amino-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]izochinolin-2-ylmethyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-10c)

Yellow amorphous solid (crystallized from chloroform/hexane) mp 126–127 °C; IR (KBr, cm^{-1}) ν_{max}: 3431, 3353, 1692, 1651, 1619, 1236, 1056, 1021, 969, 778; (signals of *trans*-**10c** were extracted from the spectra of a 93:7 mixture of *trans*-**10c** and *cis*-**10c**); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.30 (dd, 1H, J = 7.3, 1.0 Hz), 8.00 (d, 1H, J = 2.3 Hz), 7.93 (dd, 1H, J = 8.2, 1.0 Hz), 7.59 (dd, 1H, J = 8.2, 7.3 Hz), 7.29 (d, 1H, J = 2.3 Hz), 4.53–4.42 (m, 2H, *H*C5 and CH_2N), 4.31–4.25 (m, 1H, CH_2N), 4.23–4.11 (m, 4H, 2 × CH_2OP), 3.09 (ddd, 1H, J = 9.6, 7.2, 2.2 Hz, *H*C3), 2.89 (d, 3H, J = 0.8 Hz, CH_3N), 2.64 (dddd, 1H, J = 18.2, 12.5, 7.2, 7.2 Hz, H_αC4), 2.43 (dddd, 1H, J = 12.5, 11.8, 9.6, 7.2 Hz, H_βC4), 1.33 (t, 3H, J = 7.1 Hz, CH_3CH$_2$OP), 1.32 (t, 3H, J = 7.1 Hz, CH_3CH$_2$OP); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 164.41 (s, C(O)), 164.12 (s, C(O)), 145.70, 133.25, 131.78, 127.23, 126.91, 123.04, 122.85, 122.07, 121.87, 113.86, 75.17 (d, J = 7.4 Hz, C5), 63.92 (d, J = 170.9 Hz, C3), 63.26 (d, J = 6.5 Hz, CH$_2$OP), 62.74 (d, J = 6.9 Hz, CH$_2$OP), 46.76 (br s, CH$_3$N), 42.64 (s, CH$_2$N), 36.36 (s, C4), 16.77 (d, J = 5.7 Hz, CH$_3$CH$_2$OP), 16.70 (d, J = 5.7 Hz, CH$_3$CH$_2$OP); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 22.73. Anal. Calcd for $C_{21}H_{26}N_3O_6P$: C, 56.37; H, 5.86; N, 9.39; found: C, 56.42; H, 5.88; N, 9.36 (obtained on a 93:7 mixture of *trans*-**10c** and *cis*-**10c**).

4.4.4. Diethyl *trans*-5-(5-acetamido-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]izochinolin-2-ylmethyl)-2-methylisoxazolidin-3-yl-3-phosphonate (*trans*-10d)

Yellow amorphous solid (crystallized from chloroform/hexane) mp 166–168 °C; IR (KBr, cm^{-1}) ν_{max}: 3299, 2982, 1628, 1660, 1553, 1255, 1044, 973, 784; (signals of *trans*-**10d** were extracted from the spectra of a 93:7 mixture of *trans*-**10d** and *cis*-**10d**); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.81 (d, 1H, J = 2.0 Hz), 8.41 (d, 1H, J = 7.1 Hz), 8.35 (br s, N*H*), 8.22 (d, 1H, J = 2.0 Hz), 8.07 (d, 1H, J = 8.1 Hz), 7.67 (dd, 1H, J = 8.1, 7.1 Hz), 4.55–4.46 (m, 2H, *H*C5 and CH_2N), 4.29–4.13 (m, 5H, CH_2N and 2 × CH_2OP), 3.17 (ddd, 1H, J = 9.5, 7.0, 2.3 Hz, *H*C3), 2.91 (s, 3H, CH_3N), 2.72 (dddd, 1H, J = 18.0, 12.7, 7.0, 7.0 Hz, H_αC4), 2.45 (dddd, 1H, J = 12.7, 11.9, 9.5, 7.0 Hz, H_βC4), 2.30 (s, 3H, CH_3C(O)), 1.34 (t, 3H, J = 7.1 Hz, CH_3CH$_2$OP), 1.33 (t, 3H, J = 7.1 Hz, CH_3CH$_2$OP); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.74 (s, C(O)NH), 163.98 (s, C(O)), 163.47 (s, C(O)), 137.34, 133.80, 132.20, 129.62, 127.16, 124.42, 124.24, 122.37, 121.80, 121.66, 75.25 (d, J = 6.2 Hz, C5), 63.93 (d, J = 169.4 Hz, C3), 63.34 (d, J = 6.7 Hz, CH$_2$OP), 63.03 (d, J = 7.1 Hz, CH$_2$OP), 46.87 (br s, CH$_3$N), 42.70 (s, CH$_2$N), 36.27 (s, C4), 24.69 (s, CH$_3$C(O)), 16.78 (d, J = 5.5 Hz, CH$_3$CH$_2$OP), 16.71 (d, J = 5.6 Hz, CH$_3$CH$_2$OP); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 22.70. Anal. Calcd for $C_{23}H_{28}N_3O_7P$: C, 56.44; H, 5.77; N, 8.59; found: C, 56.53; H, 5.64; N, 8.67 (obtained on a 93:7 mixture of *trans*-**10d** and *cis*-**10d**).

4.5. Antiviral activity assays

The compounds were evaluated against different herpes viruses, including herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK$^-$) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strain G, varicella-zoster virus (VZV) strains Oka and YS, TK$^-$ VZV strains 07-1 and YS-R, human cytomegalovirus (HCMV) strains AD-169 and Davis as well as feline herpes virus (FHV), the poxvirus vaccinia virus (Lederle strain), *para*-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, respiratory syncytial

virus (RSV), feline coronovirus (FIPV) and influenza A virus subtypes H1N1 (A/PR/8), H3N2 (A/HK/7/87) and influenza B virus (B/HK/5/72) and human immune deficiency virus (5HVV-1 and HIV-2). The antiviral assays, other than HIV, were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey kidney cells (Vero), human epithelial cervix carcinoma cells (HeLa), Crandell-Rees feline kidney cells (CRFK), or Madin Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation (VZV) was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathicity or viral plaque formation by 50%.

4.6. Cytotoxicity assays

Cytotoxicity measurements were based on the inhibition of cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to adhere and proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of further incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required reducing cell proliferation by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Alternatively, cytotoxicity of the test compounds was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that caused a microscopically detectable alteration of cell morphology.

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