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RESEARCH ARTICLE

New phosphorylated 5-(hydroxyalkylamino)-1,3-oxazoles as potential anticancer agents

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Abstract: Eight new phosphorylated 5-(hydroxyalkylamino)-1,3-oxazoles were designed and tested for their ability to inhibit cancer cell growth. These compounds were evaluated against complete human tumor cell lines NCI-60. Only three compounds showed antitumor activity in the single dose assay, which were taken in the five dose assay. Compounds **7** and **8** showed the same average antiproliferative activity and cytotoxicity against sensitive cell lines of the general panel. However, compound **8** showed cytotoxicity to more lines than **7**. By all parameters, these compounds were more active than compounds **5**. Compounds **7** and **8** also showed high and similar antiproliferative activity in the concentration range GI_{50}: 1-6 and TGI: 6-14 μM against all subpanels. Their cytotoxicity was in the concentration range of 25-54 μM. Compound **5** showed the same activity, with the exception of the leukemia, non-small cell lung cancer and ovarian cancer subpanels against which their activity was lower. When analyzing the structure-activity, it turned out that among the phosphorylated oxazole derivatives, only compounds containing the triphenylphosphonium cation (TPP^+) in the 4th position of the oxazole ring exhibit antitumor activity. Moreover, the replacement of the phenyl radical in the 2nd position of the oxazole scaffold with a methyl radical led to the disappearance of the activity. The COMPARE algorithm reveals a high correlation of the antiproliferative activity of the tested compounds with the antitumor agents phyllantoside and chromomycin A3 in the GI_{50} vector and moderate with phyllantoside in the TGI vector. The target of all standard drugs that correlate with the cytotoxicity of the studied compounds, with the exception of didemnin, is DNA. Unlike standard compounds, synthesized active compounds carry a delocalized TPP^+, which delivers them predominantly to mitochondria due to a much more hyperpolarized potential of the mitochondrial membrane in cancer cells than in normal ones. Therefore, their anticancer activity is most likely due to a disturbation of the structural and functional state of the latter due to interference with their intrinsic protein-synthesizing apparatus of mitochondria. The data obtained allow us to consider 5-(hydroxyalkylamino)-1,3-oxazoles loaded with TPP^+ as leading compounds for further in-depth study and synthesis of new TPP^+-containing 1,3-oxazole derivatives with antitumor activity.

Keywords: phosphorylated 5-(hydroxyalkylamino)-1,3-oxazoles; synthesis; anticancer activity; COMPARE correlations.

Introduction

Today, the solution to the problem of treating cancer, which is one of the leading causes of morbidity and mortality worldwide, is considered one of the main tasks of medicine. Among the various types of treatment, chemotherapy continues to play a leading role in the treatment of cancer. However, due to the serious side effects inherent in tumor chemotherapy, as well as the development of drug resistance to them, significant efforts are being made to find and develop new drugs with increased antitumor efficacy and reduced side effects. A significant place among them belongs to 1,3-oxazole derivatives, which have a wide spectrum of antitumor activity due to their inherent multiplicity of mechanisms of action [1, 2].

The biological activity of phosphorylated oxazoles has been little studied, with the exception of compounds containing phosphonium ion, used for drug delivery to cell mitochondria. It has been shown that the antifungal activity of diethyl ethers of 1,3-oxazole-4-phosphonic acids appears

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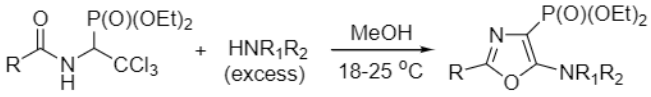
to be mediated by inhibition of aldolase II in Candida albicans [3], and antihypertensive activity of 4-phosphonyloxazoles in rats due to inhibition of phosphodiesterase III [4]. The antitumor activity of 4-triphenylphosphonium salts of 1,3-oxazol have been assessed against NCI-60 cancer cell lines, which showed high anticancer activity (GI_{50}: 0.3-1.1, TGI: 1.2-2.5 and LC_{50}: 5-6 μM) [5].

In this work, a series of novel phosphorylated 5-(hydroxylalkylamino)-1,3-oxazoles was constructed and tested for their ability to inhibit the growth of the full NCI-60 human tumor cell lines.

Results and Discussion

Chemistry

The synthesis of compounds **1-8** was carried out by known methods [6-9]. Diethyl 5-(hydroxyalkyl)amino-2-R-1,3-oxazol-4-phosphonates **1-4** were obtained by the interaction of available diethyl esters of 1-aroylamino-2,2,2-trichloroethylphosphonic acids **Ia,b** with an excess of the corresponding aminoalcohols in methanol at room temperature (Scheme 1).



I	R
a	Ph
b	4-$O_2NC_6H_4$

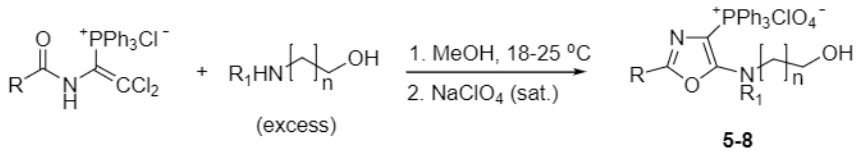
	R	R^1	R^2
1	4-$O_2NC_6H_4$	Me	$(CH_2)_2OH$
2	Ph	H	$(CH_2)_3OH$
3	Ph	H	$CH_2CH(OH)CH_2OH$
4	Ph	Me	$CH_2(CH(OH))_4CH_2OH$

Scheme 1. Synthesis of diethyl 5-(hydroxyalkyl)amino-2-aryl-1,3-oxazol-4-phosphonates **1-4.**

Phosphonium salts **5-8** were synthesized in a similar way. For this, 1-acylamino-2,2-dichloroethenylphosphonium chlorides **IIa,b** were used as starting reagents. The interaction of compounds **IIa, b** with an excess of aminoalkanol takes place in a methanol environment at 20-25 °C and leads to the formation of 2-R-5-(hydroxyalkylamino)-1,3-oxazol-4-yl(triphenyl)phosphonium chlorides. For the convenience of isolation and obtaining analytically pure samples, these substances were transformed into the corresponding perchlorates **5-8** by treating the reaction mixture with a saturated solution of sodium perchlorate (Scheme 2).

The structure of all obtained compounds was proven by means of elemental analysis, ^{1}H-, ^{13}C-, ^{31}P-NMR spectroscopy and mass spectrometry. Thus, the values of the proton signals in the ^{1}H-NMR spectra of compounds **1-8** fully correspond to the presented structures. Particular attention is drawn to the data of ^{13}C-NMR spectra, in particular, the signals of the carbon nuclei of the oxazole cycle and ethoxyl groups of products **1-4**, as well as phenyl rings in compounds **5-8**, which appear in the form of doublets due to their interaction with the nucleus of the Phosphorus atom. Thus, in phosphonates **1-4**, the signals of carbon nuclei in position 5 of the oxazole cycle appear in the region of 163.5-161.26 ppm with a spin-spin interaction constant (J) of 38.9-37.9 Hz, for C(2) in the range of 150.7-147.8 ppm with the J value of 22.4-21.9 Hz, and for C(4) in the region of 101.5-96.0 ppm and with J = 254.8-254.3 Hz. The signals of the methylene group of the diethoxyphosphoryl fragment are in the range of 62.0-61.6 ppm with spin-spin interaction constant 5.5-5.0 Hz, and the methyl group at 16.2 ppm with J 6.5-6.0 Hz.

Replacement of the 1,3-oxazole ring of the diethoxyphosphoryl group in position 4 with a more electron-accepting triphenylphosphonium does not significantly affect the position of the signals of the Carbon nuclei in positions 2 and 5, however, a shift of the signals of the Carbon C(4) nuclei to a stronger field region of the spectrum is observed. Thus, the signals of Carbon C(5) nuclei appear in the range of 165.31-164.0 ppm (29.8-27.6 Hz), C(2) at 153.3-152.8 ppm (21.3-20.5 Hz), and C(4) at 85.84-81.62 ppm with spin-spin interaction constant 154.8-153.3 Hz. The signals of the phenyl fragment of the triphenylphosphonium residue of oxazoles **5-8** in the ^{13}C NMR spectra are in the region: C(4) 135.45-135.31 ppm (3.0-2.9 Hz), C(3) 134.76-134.51 ppm (11.0-10.5 Hz), C(2)



II	R
a	Me
b	Ph

	R	R^1	n
5	Ph	H	1
6	Me	H	1
7	Ph	Me	1
8	Ph	H	2

Scheme 2. Synthesis of 5-(hydroxyalkyl)amino-2-R-1,3-oxazol-4-(triphenyl)phosphonium perchlorates **5-8**.

130.63-130.58 ppm (13.2-12.5 Hz) and C(1) 120.54-119.40 ppm (93.9-93.2 Hz). Signals of phosphorus nuclei in the ^{31}P-NMR spectra of compounds **1**-**8** are fixed in the range of 14.3-10.5 ppm.

In the IR spectra of compounds **1**-**8**, intense absorption bands of C=N groups of the oxazole cycle were found in the region of 1628-1605 cm^{-1} and 1601-1584 cm^{-1}. In phosphonates **1**-**4**, the absorption bands of the P=O group are fixed at 1245-1200 cm^{-1}, and those of the P-O-C bonds at 1049-1016 cm^{-1} and 976-961 cm^{-1}. The absorption bands of perchlorate anion in the range of 1110-1106 cm^{-1} are observed in phosphonium salts **5**-**8**.

Biology

One dose assay

According to the mean growth inhibition percent of the total panel, indicated in brackets, the tested compounds according to the increase in activity are arranged in a row: **1** = **2** = **3** = **4** = **6** (≤6) < **5** (92) < **7** = **8** (98). Hence, only compounds **5, 7** and **8** showed antitumor activity, complete data for which are presented in Table S1. The data of statistical analysis of the antitumor activity of compounds **5, 7** and **8** by subpanels are presented in Table 1.

Table 1. Average growth inhibition of the NCI-60 cancer cell line subpanels by compounds **5**, **7** and **8** in one dose assay.

Subpanel	Compound		
	5	**7**	**8**
Leukemia	99±6	98±7	100±7
NSCLC	84±8	97±5	92±6
Colon cancer	96±16	102±14	103±13
CNS cancer	90±3	99±7	95±2
Melanoma	112±16	124±12	126±13
Ovarian cancer	83±15	83±15	84±14
Renal cancer	48±12	69±12	66±10
Breast cancer	112±8	116±9	102±11

The compounds added at a concentration ($1 \cdot 10^{-5}$ M) and the culture incubated for 48 h. The number reported for the one-dose assay is growth inhibition (%) relative to the no-drug control, and relative to the time zero number of cells. NSCL - Non-Small Cell Lung cancer. Data represent as mean ± SE, %.

Compounds **5**, **7** and **8** showed equally effective growth inhibition with the highest and lowest activity against the melanoma and renal cancer subpanels, respectively. According to the results of statistical processing of the obtained data, these compounds were taken for a five dose assay.

Five dose assay

Total panel

The calculated parameters of the antitumor activity of the tested compounds against the cell lines of the total panel are presented in the Table S2, and the results of the statistical analysis in the Table 2.

Table 2. Average values of parameters of anticancer activity and the number of cell lines sensitive to the action of the tested compounds against cell lines of the total panel in a five-dose analysis.

Parameter	Compound		
	5	**7**	**8**
GI_{50}	5.37±0.73 (59)	2.85±0.38 (59)	3.26±0.38 (59)
TGI	21.43±2.37 (56)	13.02±1.24 (59)	11.80±0.84 (59)
LC_{50}	51.23±3.55 (37)	40.73±2.50 (45)	40.70±2.32 (52)

Parameter values are expressed in μM (M±m). Numbers in parentheses indicate the number of sensitive lines, i.e., for which the parameter values are less than 100 μM.

Compounds **7** and **8** showed the same average antiproliferative activity and cytotoxicity against sensitive cell lines of the total panel. However, compound **8** showed cytotoxicity to more lines than **7**. In all respects, these compounds were statistically significantly more active than compounds **5**.

A similar pattern was noted for the dose distribution of the activity of the tested compounds (Figures S1-S3). At concentrations up to 10 μM, compounds **7** and **8** showed 50% inhibition in 95 and 92% of cell lines, respectively, versus 83% for compound **5.** Cytostatic activity of compounds **7** and **8** was observed against 92 and 96% of cells at concentrations up to 30 μM, then as this parameter for compound **5** was 76%. Compound **8** at concentrations up to 100 μM was cytotoxic to 86% of cells, while compounds **7** and **5** were cytotoxic against 72 and 60%, respectively.

Subpanels

The data of statistical analysis of the antitumor activity of compounds **5, 7** and **8** by subpanels are presented in Table 3. Parameter values are expressed in μM (M±m). The numbers in parentheses indicate the number of sensitive lines, i.e., for which the parameter values are ≤ 100 μM.

Compounds **7** and **8** demonstrated high and identical antiproliferative activity against all subpanels in all calculated parameters characterizing their antitumor activity for exception of NCI/ADR-RES ovarian cancer cell line (Table S2). Thus, the value of 50% inhibition of cell growth for these compounds was in the concentration range of 1-6 μM, and for cytostatic activity was in the range of 6-14 μM.

Table 3. Average antitumor activity parameters of compounds **5, 7** and **8** against the NCI-60 cancer subpanels in five dose assay.

Subpanel	5			7			8		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Leukemia	2.9±0.7 (6)	19.3±4.1 (6)	70.1±8.3 (2)	1.5±0.4 (6)	9.5±1.9 (6)	61.3±11.0 (3)	1.9±0.5 (6)	11.1±1.8 (6)	53.7±10.1 (5)
NSLC	7.1±2.0 (9)	30.7±6.5 (8)	44.6±4.1 (3)	2.7±0.4 (9)	13.0±3.0 (9)	41.9±4.0 (7)	3.5±0.8 (9)	13.0±1.8 (9)	42.4±2.4 (9)
Colon cancer	7.3±3.8 (7)	19.8±5.1 (6)	59.1±12.3 (4)	3.9±1.6 (7)	16.2±3.6 (7)	40.3±10.3 (5)	4.0±1.6 (7)	13.8±2.9 (7)	44.8±4.8 (5)
CNS cancer	4.6±1.3 (6)	15.8±2.4 (6)	60.7±7.0 (6)	2.4±0.3 (6)	10.6±1.3 (6)	37.9±3.6 (6)	2.6±0.4 (6)	10.2±1.3 (6)	36.3±4.0 (6)
Melanoma	2.7±0.3 (9)	9.7±1.8 (9)	37.4±7.7 (9)	1.8±0.2 (9)	8.6±1.1 (9)	30.6±4.0 (9)	2.1±0.2 (9)	8.3±1.6 (9)	25.0±4.1 (8)
Ovararian cancer	3.5±0.4 (6)	24.2±8.0 (6)	60.9±9.9 (4)	2.2±0.4 (6)	13.3±3.1 (6)	48.7±5.8 (4)	2.4±0.3 (6)	10.9±1.6 (6)	36.8±2.3 (5)
Renal cancer	11.3±2.5 (8)	39.8±9.8 (8)	49.8±8.9 (4)	6.4±2.0 (8)	24.5±5.7 (8)	46.2±7.7 (5)	6.7±1.7 (8)	19.0±3.2 (8)	51.9±7.1 (8)
Breast cancer	2.2±0.3 (6)	8.6±1.8 (5)	45.7±15.6 (4)	1.3±0.3 (6)	6.6±1.4 (6)	37.0±12.3 (5)	2.1±0.3 (6)	6.1±1.0 (6)	34.4±11.4 (5)

Their cytotoxicity was in the concentration range of 25-54 μM. Compound **5** showed the same activity with the exception of leukemia, NSCL and ovarian cancer, against which their potency was lower.

When analyzing the structure-activity, it can be seen that among the phosphorylated oxazole derivatives, only compounds containing a triphenylphosphonium cation (TPP^+) in the 4th position of the oxazole ring exhibit antitumor activity. Moreover, the replacement of the phenyl radical in the 2nd position by the methyl one (compound **6**) led to the elimination of the activity. A significant effect on the activity of TPP^+ oxazole derivatives by the presence of an aromatic substituent in the 2nd position was also shown by previously obtained data [5]. In this work, it was shown that derivatives in which aromatic radicals (phenyl, aminophenyl, tolyl or methylphenylsulfonyl) in this position of oxazole were replaced by methyl radical were either inactive or weakly active against NCI-60 cell lines. The activity of compound **5** against the total panel was inferior in all parameters to compounds **7** and **8**, the only difference between which was the substitution in the 5th position of 2- (hydroxyethyl)(methyl)amine (**7**) or 3-hydroxypropylamine (**8**) on 2-hydroxyethylamine. Apparently, this substitution made some contribution to the elimination of the activity of compound **6**. The essential significance of the functionalization of the 5th position is confirmed by the data given in the above cited work. Thus, tested analogues of compounds **7** and **8** containing in this position aromatic radicals (methylphenylsulfanyl, methylphenylamine or phenylamine) instead hydroxyalkylamines showed higher antitumor potency.

Compare correlation

GI_{50}, TGI and LC_{50} vectors were analyzed by COMPARE, generating correlation coefficients for the compounds with known mechanisms of anticancer activity presented in public databases to suggest their likely molecular mechanisms or specific targets. The quantitative evaluation of the obtained results was carried out according to the Chaddock scale [10].

Reported mechanisms of the standard drugs correlating with GI50 and TGI vectors of tested compounds

The COMPARE algorithm reveals a high correlation of antiproliferative potency of tested compounds with anticancer agents phyllanthoside and chromomycin A3 by the GI_{50} vector, and moderate with phyllanthoside by the TGI vector. Chromomycin A3 is a GC-specific DNA ligand which inhibits transcription selectively inhibiting the DNA-directed RNA polymerase reaction. Does not intercalate [11, 12]. In addition, it is a selective Sp1 inhibitor that specifically suppresses the expression of the anti-apoptotic gene associated with the Sp1 protein and induces caspase-dependent apoptosis [13]. Phyllanthoside suppresses protein synthesis in cells by inhibiting the elongation mechanism during translation [14, 15] that correlate with its effects on cellular protein synthesis [16].

The presence of a strong correlation of the test compounds with phyllantoside and chromomycin A3 indicates the possible involvement of molecular mechanisms inherent in these drugs in the antiproliferative effect of the test compounds.

Table 4. Standard agent COMPARE correlations for compounds **5, 7** and **8** tested by five dose assay

Compd.	GI_{50}	TGI	LC_{50}
5	Phyllanthoside (0.75) Chromomycin A3 (0.72)	Phyllanthoside (0.68)	Trimelamol (0.67), Actinomycin D (0.65)
7	Chromomycin A3 (0.81) Phyllanthoside (0.78)	Phyllanthoside (0.67)	Didemnin B (0.69) Morpholino doxorubicin (0.67) Chromomycin A3 (0.65)
8	Chromomycin A3 (0.78) Phyllanthoside (0.74)	Phyllanthoside (0.67)	MX2 HCl (0.58), daunorubicin, doxorubicin, cyanomorpholino doxorubicin, actinomycin D, and tetraplatin (0.56)

Reported mechanisms of the standard drugs correlating with LC50 vector of tested compounds

The target of all standard drugs that correlate with the cytotoxicity of the studied compounds, with the exception of didemnine is DNA. These drugs act primarily as DNA intercalating and alkylating agents, interfering with DNA metabolism and RNA production. These process itself results in a distortion of the DNA conformation that causes the inhibition of topoisomerase II. Cytotoxicity is primarily due to inhibition of topoisomerase II after the enzyme induces a break in DNA, preventing religation of the break and leading to cell death [17-22].

Didemnin B like Phyllanthoside inhibits the elongation of mRNA translation on the ribosome during protein synthesis, and induces cell apoptosis. Although in some cancer cell types, apoptosis is only induced by concentrations at least one order of magnitude higher than needed to inhibit translation [23, 24]. The moderate correlation of the cytotoxicity vector of the tested compounds with that of the above agents, on the one hand, does not provide sufficient grounds for classifying their mechanisms as those of the tested compounds, and on the other hand, the absence of drugs with other mechanisms of action in this list increases the probability of their inclusion.

It should be noted that these compounds contain bulky TPP^+, which prevents DNA intercalation, which, however, does not exclude the possibility of their binding to certain regions of nucleic acids, like didemnin, phyllantoside, trimelamol, and chromomycin A3, preventing the processes of transcription and translation in the cell. In addition, a moderate correlation may be due to the fact that the subcellular target of TPP^+-loaded compounds is mitochondria, since the mitochondrial membrane potential in cancer cells (-220 mV, negative inside) is more hyperpolarized than in normal cells (-140 mV), which creates a 100-1000-fold concentration gradient of such compounds in favor of mitochondria [25]. That is, they predominantly interact with their intrinsic mitochondrial protein-synthesizing apparatus, and not with the general cellular one, which cannot but affect the results of a COMPARE analysis. Therefore, the functionalization of known antitumor agents with TPP^+ is often used for their selective accumulation in the mitochondria of cancer cells [26]. It helps to increase selectivity, anticancer activity, overcome drug resistance and reduce side effects [27, 28]. In addition, TPP^+ is not an inert transporter, and can promote proton leakage and uncoupling of oxidative phosphorylation, which reduces the efficiency of ATP generation [29]. Therefore, unlike standard compounds, oxazole TPP^+ derivatives delivered to mitochondria are likely to selectively disrupt mitochondrial protein synthesis, which results in excessive production of reactive oxygen species, inhibition of cell proliferation, release of cytochrome c, and apoptosis through the mitochondrial pathway [30, 31].

From this standpoint, the tested 5-(hydroxyalkylamino)-1,3-oxazoles loaded with TPP^+ should be considered as promising compounds with high potential antitumor activity and can be taken for further functionalization in order to increase their antitumor activity and reduce toxicity to normal cells.

Conclusions

Eight new phosphorylated 5-(hydroxyalkylamino)-1,3-oxazoles were tested for their ability to inhibit cancer cell growth. These compounds were evaluated against complete human tumor cell lines NCI-60. Structura-active analysis showed that among the phosphorylated oxazole derivatives, only compounds containing the triphenylphosphonium cation in the 4th position of the oxazole ring exhibit antitumor activity against all the studied cancer cell lines. Moreover, among them, only compounds containing a phenyl radical in the 2nd position of the oxazole skeleton turned out to be active. Compounds **7** and **8** showed strong and similar antiproliferative activity against all subpanels. Compound **5** showed the same activity, except for the leukemia, NSCL and ovarian cancer subpanels where their efficacy was lower. A COMPARATIVE analysis showed the highest GI_{50} correlation of compound **5** with phyllantoside, and compounds **7** and **8** with chromomycin A3. The cytostatic activity of all active compounds moderately correlates with drugs whose cytotoxicity is mediated by interaction with nucleic acids, which leads to disruption of the protein-synthesizing function of the cell. Unlike standard compounds, test compounds containing TPP^+ accumulate predominantly in mitochondria. Therefore, their anticancer activity is most likely due to a violation of the structural and functional state of the latter

due to interference with their intrinsic protein-synthesizing apparatus of mitochondria. The data obtained allow us to consider the studied compounds as leading ones for further in-depth study and synthesis of new TPP^+-containing 1,3-oxazole derivatives with antitumor activity.

Notes

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Disclaimer. This material should not be interpreted as representing the viewpoint of the viewpoint of the US National Institutes of Health, or the National Cancer Institute.

Experimental section

Chemistry

^1H, ^{13}C, ^{31}P NMR spectra were obtained with a Bruker AVANCE DRX-500 spectrometer (TMS as internal reference or 85% phosphoric acid as external reference) in DMSO-d_6. IR spectra were recorded with a Vertex 70 spectrometer in KBr pellets or film. LC-MS spectra were recorded on an LC-MS system-HPLC Agilent 1100 Series equipped with a diode array detector Agilent LC\MSD SL. Parameters of LC-MS analysis: Zorbax SB-C18 column (1.8 lm, 4.6-15 mm, PN 821975-932), solvent water-acetonitrile mixture (95:5), 0.1% of aqueous trifluoroacetic acid; eluent flow 3 mL min1; injection volume 1 lL; UV detection at 215, 254, 265 nm; chemical ionization at atmospheric pressure (APCI), scan range m/z 80-1000. Elemental analysis was carried out in the Analytical Laboratory of the Institute of Bioorganic and Petrochemistry of the National Academy of Sciences of Ukraine by manual methods. The carbon and hydrogen contents were determined using the Pregl gravimetric method, while nitrogen was determined using the Duma's gasometrical micromethod. Chlorine content was determined by the mercurometric method, phosphorus content was determined by the colorimetric method. M.p. were determined with a Fisher-Johns apparatus and are uncorrected. Reactions and purity of the products were monitored by thin-layer chromatography on DC-Fertigfolien ALUGRAM Xtra SIL G/UV_{254} plates using 9:1 (v/v) chloroform–methanol as eluent. All reagents and solvents were purchased from Aldrich and used as received.

*General procedure for preparing diethyl 5-(hydroxyalkyl)amino-2-R-1,3-oxazol-4-phosphonates (**1-3**).*

To a solution of 0.001 mol of compounds **Ia,b** in 50 mL of methanol was added 0.045 mol of aminoalkanole. The mixture was stirred for 12-24 h at a temperature of 18-25 °C. After the solvent removal in vacuo, the residue treated with 20 ml distilled water and 20 mL of *tert*-butyl methyl ether. Organic phase dried over sodium sulfate. The solvent was removed in a vacuum, and the reaction products **1–3** were purified by column chromatography (dichloromethane – methanol, 95:5)

*Diethyl 5-((hydroxyethyl)(methyl)amino)-2-(4-nitrophenyl)-1,3-oxazol-4-phosphonate (**1**)* [6].

Yeild: 73%; mp 144-147 °C. IR (KBr): ν = 3423 (OH), 1612, 1591 (C=N), 1508, 1343 (N–O), 1245 (P=O), 1049, 969 (P-O-C). ^1H NMR (500 MHz, DMSO-d_6): δ 8.33 (d, 2H, 4-$O_2NC_6H_4$, J 8.8 Hz), 8.03 (d, J 8.3 Hz, 2H, 4-$O_2NC_6H_4$,), 4.82 (br s, 1H, OH), 4.11-4.01 (m, 4H, $2OCH_2CH_3$), 3.73-3.62 (m, 4H, $2CH_2$), 3.27 (s, 3H, CH_3), 1.28 (t, ^3J 7.0 Hz, 6H, $2OCH_2CH_3$). ^{13}C NMR (125.69 MHz, DMSO-d_6): δ 161.8 (d, J_{PC} 37.9 Hz, C^5 oxazole), 147.8 (d, J_{PC} 21.9 Hz, C^2 oxazole), 147.2, 132.0, 125.6, 124.5 (4-$O_2NC_6H_4$), 101.5 (d, J_{PC} 254.3 Hz, C^4 oxazole), 62.0 (d, $^2J_{PC}$ 5.5 Hz, $POCH_2CH_3$), 58.7, 54.6, 38.4, 16.2 (d, $^3J_{PC}$ 6.5 Hz, $POCH_2CH_3$). ^{31}P NMR (202.38 MHz, DMSO-d_6): δ 12.7. LCMS: 400[M + 1]. Anal. Calcd. for $C_{16}H_{22}N_3O_7P$ (399.34): C, 48.12; H 5.51; N 10.52; P 7.76. Found: C, 48.01; H, 5.62; N, 10.50; P, 7.73.

*Diethyl 5-((hydroxypropyl)amino)-2-(phenyl)-1,3-oxazol-4-phosphonate (**2**)* [7].

Yeild: 72%; mp 61-63 °C. IR (KBr): ν = 3286 (OH, NH), 1628, 1585 (C=N), 1200 (P=O), 1020, 975 (P-O-C). ^1H NMR (500 MHz, DMSO-d_6): δ 7.83 (d, ^3J 7.4 Hz, 2H, C_6H_5), 7.53-7.35 (m, 3H, C_6H_5), 6.70-6.63 (m, 1H, NH), 4.63 (br s, 1H, OH), 4.09-3.93 (m, 4H, $2OCH_2CH_3$), 3.58-3.50 (m, 2H, CH_2OH), 3.50-3.41 (m, 2H, NCH_2), 1.80-1.70 (m, 2H, CH_2), 1.31-1.17 (m, 6H, $2OCH_2CH_3$). ^{13}C NMR (125.69 MHz, DMSO-d_6): δ 163.1 (d, $^2J_{PC}$ 38.9 Hz, C^5 oxazole), 150.6 (d, $^3J_{PC}$ 21.9 Hz, C^2 oxazole), 129.6, 129.1, 127.5, 124.9 (C_6H_5), 98.9 (d, $^1J_{PC}$ 254.8 Hz, C^4 oxazole), 61.6 (d, $^2J_{PC}$ 5.0 Hz, $POCH_2CH_3$), 58.4, 40.4, 32.7, 16.2 (d, $^3J_{PC}$ 6.0 Hz, $POCH_2CH_3$). ^{31}P NMR (202.38 MHz, DMSO-d_6): δ 13.5. LCMS: [M + 1]: 355. Anal. Calcd. for $C_{16}H_{23}N_2O_5P$ (354.34): C, 54.23; H, 6.54; N, 7.91; P, 8.74. Found: C, 54.39; H, 6.49; N, 8.27; P, 8.53.

Diethyl 5-((2,3-dihydroxypropyl)amino)-2-(phenyl)-1,3-oxazol-4-phosphonate (3) [6].

Yeild: 63%; mp 110-112 °C. IR (KBr): ν = 3334 (OH, NH), 1626 br (C=N), 1215 (P=O), 1020, 976 (P-O-C). ^1H NMR (500 MHz, DMSO-d_6): δ 7.83 (d, ^3J 7.1 Hz, 2H, C_6H_5), 7.54-7.41 (m, 3H, C_6H_5), 6.49 (br s, 1H, NH), 5.02 (br s, 1H, OH), 4.72 (br s, 1H, OH), 4.10-3.92 (m, 4H, $2OCH_2CH_3$), 3.70-3.61 (m, 1H, CH), 3.57-3.49 (m, 1H, CH), 3.44-3.37 (m, 1H, CH), 3.36-3.24 (m, 2H, CH), 1.26-1.21 (m, 6H, $2OCH_2CH_3$). ^{13}C NMR (125.69 MHz, DMSO-d_6): δ 163.5 (d, $^2J_{PC}$ 38.9 Hz C^5 oxazole), 150.7 (d, $^3J_{PC}$ 22.4 Hz, C^2 oxazole), 129.6, 129.1, 126.6, 124.9 (C_6H_5), 96.0 (d, $^1J_{PC}$ 254.8 Hz, C^4 oxazole), 70.2, 63.5, 61.6 (d, $^2J_{PC}$ 5.0 Hz, $POCH_2CH_3$), 46.1, 16.2 (d, $^3J_{PC}$ 6.5 Hz, $POCH_2CH_3$). ^{31}P NMR (202.38 MHz, DMSO-d_6): δ 13.6. LCMS: [M + 1]: 371. Anal. Calcd. for $C_{16}H_{23}N_2O_6P$

(370.34): C, 51.89; H, 6.26; N, 7.56; P, 8.36. Found: C, 52.00; H, 6.10; N, 7.40; P, 8.68.

*Diethyl 5-{methyl-[(2R,3S,4S,5S)-2,3,4,5,6-pentahydroxyhexyl]amino}-2-phenyl-1,3-oxazol-4-phosphonate (**4**)* [8].

To a solution of 0.001 mol of compound **IIb** in 50 mL of methanol was added 0.0045 mol of N-methyl-D-glucamine. The mixture was stirred for 6-12 h at a temperature of 18-25 °C. The precipitated N-methyl-D-glucamine hydrochloride was filtered off. After the solvent removal, 20 mL of tetrahydrofuran was added to oily residue. The precipitate was filtered off, the solvent was removed in a vacuum, and the reaction product **4** were analyzed without further purification. Yeild: 90%; $[\alpha]_D$ +23.30°; Oil. IR (film): ν = 3346 (O-H), 1605, 1584 (C=N),, 1205 (P=O), 1016, 961 (P-O-C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.83 (d, ^3J 7.1 Hz, 2H, C_6H_5), 7.49-7.43 (m, 3H, C_6H_5), 4.93 (br s, 1H, OH), 4.54 (br s, 1H, OH), 4.45 (br s, 2H, OH), 4.35 (br s, 1H, OH), 4.10-4.00 (m, 4H, $2OCH_2CH_3$), 3.98-3.93 (m, 1H, CH), 3.69-3.47 (m, 7H, $2CH_2$, 3CH), 3.24 (s, 3H, NCH_3), 1.26 (t, ^3J 7.1 Hz, 6H, $2OCH_2CH_3$,). ^{13}C NMR (125.69 MHz, DMSO-d_6): δ 161.26 (d, $^2J_{PC}$ 38.4 Hz, C^5 oxazole), 149.54 (d, $^3J_{PC}$ 22.4 Hz, C^2 oxazole), 129.51, 129.07, 126.66, 124.91 (C_6H_5), 98.67 (d, $^1J_{PC}$ 254.6 Hz, C^4 oxazole), 71.81, 71.56, 70.89, 70.24, 63.52, 61.89 (d, $^2J_{PC}$ 5.0 Hz, $POCH_2CH_3$), 55.31, 39.08, 16.22 (d, $^3J_{PC}$ 6.5 Hz, $POCH_2CH_3$). ^{31}P NMR (202.38 MHz, DMSO-d_6): δ 14.30. LCMS: 475 [M + 1]. Anal. Calcd. for $C_{20}H_{31}N_2O_9P$ (474.44): C, 50.63; H, 6.59; N, 5.90; P, 6.59. Found: C, 50.91; H, 6.48; N, 5.99; P, 6.60.

*General procedure for preparing 2-R-5-[(2-hydroxyalkyl)amino]-1,3-oxazol-4-yltriphenylphosphonium perchlorates (**5, 6, 8**).*

To a solution of 0.001 mol of compound **II a**, **b** in 50 ml of methanol was added 0.0035 mol of alkanolamine, the mixture was stirred for 8 h at 18-25 °C. The solvent was removed *in vacuo* to 1/3 volume, 30 ml of methyl *tert*-butyl ether was added, stirred and left for 0.5 h, the solvents were decanted, the oily residue was kept *in vacuo* until the solvents were completely removed. 40 ml of water was added, filtered and 5 ml of saturated aqueous $NaClO_4$ was added to the aqueous solution. The precipitate was filtered and the compounds **5**-**8** were purified by recrystallization from methanol.

*[5-(2-Hydroxyethyl)amino]-2-phenyl-4-oxazolyl]triphenylphosphonium perchlorate (**5**)* [9].

Yeild: 81%; colorless solid; mp 180-182 °C. IR (KBr): ν = 3437, 3274, 1622, 1584, 1437, 1110, 1063, 725, 690, 621, 566, 518 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.97-7.88 (m, 3H, C_6H_5), 7.85-7.72 (m, 14H, C_6H_5), 7.55-7.46 (m, 3H, C_6H_5), 7.00 (t, 1H, J 5.3 Hz, NH), 4.85 (t, 1H, J 5.3 Hz, OH), 3.49-3.45 (m, 2H, CH_2), 3.41-3.35 (m, 2H, CH_2). ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 164.37 (d, J_{PC} 29.3 Hz, C^5 oxazol), 153.30 (d, J_{PC} 20.5 Hz, C^2 oxazol), 135.42 (d, J_{PC} 2.9 Hz, C^4 C_6H_5), 134.64 (d, J_{PC} 11.0 Hz, C^3 C_6H_5), 130.91, 130.63 (d, J_{PC} 13.2 Hz, C^2 C_6H_5), 129.65, 126.24, 125.74, 119.43 (d, J_{PC} 93.9 Hz, C^1 C_6H_5), 84.15 (d, J_{PC} 153.3 Hz, C^4 oxazol), 60.22 (CH_2), 46.52 (CH_2). ^{31}P NMR (202.38 MHz, DMSO-d_6): δ 10.70. LCMS: [M-M(ClO_4^-)]$^+$: 465.2. Anal. Calcd. for $C_{29}H_{26}ClN_2O_6P$ (564.95): C, 61.65; H, 4.64; Cl, 6.28; N, 4.96; P, 5.48. Found: C, 61.93; H, 4.71; Cl, 6.37; N, 5.29; P, 5.63.

*[5-(2-Hydroxyethyl)amino]-2-methyl-4-oxazolyl]triphenylphosphonium perchlorate (**6**)* [9].

Yeild: 67%; colorless solid; mp 163-165 °C. IR (KBr): ν = 3507, 3352, 1626, 1601, 1438, 1107, 724, 690, 621, 583, 546, 522 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.91-7.84 (m, 3H, C_6H_5), 7.78-7.66 (m, 12H, C_6H_5), 6.63 (t, 1H, J 5.6 Hz, NH), 4.74 (t, 1H, J 5.3 Hz, OH), 3.38-3.30 (m, 2H, CH_2), 3.20 (m, 2H, NCH_2), 2.33 (s, 3H, CH_3). ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 164.37 (d, J_{PC} 29.3 Hz, C^5 oxazol), 153.29 (d, J_{PC} 21.3 Hz, C^2 oxazol), 135.31 (d, J_{PC} 2.9 Hz, C^4 C_6H_5), 134.51 (d, J_{PC} 11.0 Hz, C^3 C_6H_5), 130.58 (d, J_{PC} 12.5 Hz, C^2 C_6H_5), 119.60 (d, J_{PC} 93.9 Hz, C^1 C_6H_5), 81.62 (d, J_{PC} 154.8 Hz, C^4 oxazol), 60.14 (CH_2), 46.36 (CH_2), 13.85 (CH_3). ^{31}P NMR (202.38 MHz, DMSO-d_6): δ 10.50. LCMS: [M-M(ClO_4^-)]$^+$: 404.2. Anal. Calcd. for $C_{24}H_{24}ClN_2O_6P$ (502.88): C, 57.32; H, 4.81; Cl, 7.05; N, 5.57; P, 6.16. Found: C, 57.51; H, 5.02; Cl, 7.13; N, 5.89; P, 6.07.

*[5-(2-Hydroxypropyl)amino]-2-phenyl-4-oxazolyl]triphenylphosphonium perchlorate (**8**)* [9].

Yeild: 77%; colorless solid; mp 194-196 °C. IR (KBr): ν = 3509, 3263, 1623, 1584, 1437, 1371, 1163, 1106, 1050, 762, 722, 687, 563, 515 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.94-7.87 (m, 3H, C_6H_5), 7.85-7.74 (m, 12H, C_6H_5), 7.66-7.58 (m, 2H, C_6H_5), 7.53-7.47 (m, 3H, C_6H_5), 7.23 (t, J 4.7 Hz, 1H, NH), 4.55 (br s, 1H, OH), 3.43-3.13 (m, 4H, $2CH_2$), 1.67-1.56 (m, 2H, CH_2). ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 164.00 (d, J_{PC} 29.3 Hz, C^5 oxazol), 152.86 (d, J_{PC} 20.5 Hz, C^2 oxazol), 135.33 (d, J_{PC} 2.9 Hz, C^4 C_6H_5), 134.69 (d, J_{PC} 11.0 Hz, C^3 C_6H_5), 130.82, 130.58 (d, J_{PC} 13.2 Hz, C^2 C_6H_5), 129.64, 126.30, 125.67, 119.40 (d, J_{PC} 93.2 Hz, C^1 C_6H_5), 83.79 (d, J_{PC} 154.1 Hz, C^4 oxazol), 58.54, 41.46, 32.57. ^{31}P NMR (202.38 MHz, DMSO-d_6): δ 10.62. LCMS: [M-M(ClO_4^-)]$^+$: 479.2. Anal. Calcd. for $C_{30}H_{28}ClN_2O_6P$ (578.98): C, 62.23; H, 4.87; Cl, 6.12; N, 4.84; P, 5.35. Found: C, 62.49; H, 4.93; Cl, 6.37; N, 5.11; P, 5.21.

*5-[(2-hydroxyethyl)(methyl)amino]-2-phenyl-1,3-oxazol-4-yltriphenylphosphonium perchlorate (**7**)* [9].

To a solution of 0.001 mol of compound **IIb** in 50 ml of methanol at -20 °C was added 0.26 g (0.0035 mol) of *N*-methyl monoethanolamine, the mixture was gently heated to 0-5 °C and maintained at this temperature for 8 hours. The solvent was removed in vacuo to 1/3 volume, 30 ml of methyl *tert*-butyl ether was added, stirred and left for 0.5 h, the solvents were decanted, the oily residue was kept in vacuo until the solvents were completely removed. 40 ml of water was added, filtered and 5 ml of saturated aqueous $NaClO_4$ was added to the aqueous solution. The precipitate

was filtered and the compound **7** were purified by recrystallization from methanol. Yeild: 61%; colorless solid; mp 218-220 °C. IR (KBr): ν = 3494, 1624, 1603, 1585, 1567, 1440, 1396, 1108, 1070, 1021, 992, 755, 723, 692, 620, 560, 518 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 7.96-7.84 (m, 9H, C_6H_5), 7.83-7.72 (m, 8H, C_6H_5), 7.54-7.46 (m, 3H, C_6H_5), 4.89 (t, J 4.9 Hz, 1H, OH), 3.51-3.45 (m, 2H, CH_2), 3.34 (s, 3H, CH_3), 3.27 (t, J 5.4 Hz, 2H, CH_2). ^{13}C NMR (125.69 MHz, DMSO-d_6): δ 165.31 (d, J_{PC} 27.6 Hz, C^5 $_{oxazol}$), 152.80 (d, J_{PC} 21.1 Hz, C^2 $_{oxazol}$), 135.45 (d, J_{PC} 3.0 Hz, C^4 C_6H_5), 134.76 (d, J_{PC} 10.5 Hz, C^3 C_6H_5), 131.00, 130.63 (d, J_{PC} 13.0 Hz, C^2 C_6H_5), 129.62, 126.10, 125.69, 120.54 (d, J_{PC} 93.4 Hz, C^1 C_6H_5), 85.84 (d, J_{PC} 153.6 Hz, C^4 $_{oxazol}$), 58.26, 55.71. ^{31}P NMR (202.38 MHz, DMSO-d_6): δ 13.97. LCMS: $[M\text{-}M(ClO_4^-)]^+$: 479.2. Anal. Calcd. for $C_{30}H_{28}ClN_2O_6P$ (578.98): C, 62.23; H, 4.87; Cl, 6.12; N, 4.84; P, 5.35. Found: C, 62.41; H, 5.06; Cl, 6.09; N, 4.99; P, 5.18.

Biology

In vitro anticancer screening of the tested compounds

One-dose assay

Synthesized compounds **1-8** were submitted to National Cancer Institute (NCI), Bethesda, Maryland, US, under the Developmental Therapeutic Program DTP. The cell line panel engaged a total of 60 different human tumor cell lines derived from nine cancer types, including lung, colon, melanoma, renal, ovarian, brain, leukemia, breast and prostate.

Primary *in vitro* one-dose anticancer screening was initiated by cell inoculating of each 60 panel lines into a series of standard 96-well microliter plates at 5000-40000 cells/well in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine (day 0), and then preincubated in absence of drug at 37 °C and 5% CO_2 for 24 h. Test compounds were then added into the plates at one concentration of 10^{-5} M (day 1), followed by incubation for a further 48 h at the same conditions. Then the media was removed, the cells were fixed *in situ*, washed, and dried (day 3). The sulforhodamine B assay was used for cell density determination, based on the measurement of cellular protein content. After an incubation period, cell monolayers were fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye was removed by washing repeatedly with 1% (vol/vol) acetic acid. The bound stain was resolubilized in 10 mM Tris base solution and measured spectrophotometrically on automated microplate readers for OD determination at 510 nm.

Five-dose assay

Compounds which exhibit significant growth inhibition in the one dose screen were evaluated against the 60 cell panel at five concentration levels (0.01, 0.1, 1, 10 and 100 μM). The outcomes were used to create three dose-response parameters (GI_{50}, TGI and LC_{50}) calculated for each cell line. The GI_{50} value (50% growth inhibition) is measure of the sensitivity of a cell to the effect of the drug, and corresponds to the concentration of the compound causing 50% decrease in net cell growth. The TGI (total growth inhibition) refers to the maximum effect of a drug and is the concentration of the study drug that causes total inhibition of cell growth. The LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h.

The three dose-response parameters GI_{50}, TGI and LC_{50} were calculated for each experimental compound. Data calculations were performed according to the method described by the NCI/NIH Development Therapeutics Program https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm.

Growth inhibition of 50% (GI_{50}) is calculated from: $[(T\text{-}T_0)/(C\text{-}T_0)]\times100 = 50$. The TGI is calculated from: $100\times(T\text{-}T_0)/(C\text{-}T_0) = 0$. Thus, the TGI signifies a cytostatic effect. The LC_{50}, which means a cytotoxic effect, is calculated as: $[(T\text{-}T_0)/T_0]\times100 = -50$, where: T_0 is the cell count at day 0; C is the vehicle control (cell count without drug), and T is the cell count at the test concentration of drug at the end of the incubation period.

Data analysis

Statistical Data analysis

Statistical analysis of the results and the histograms was performed by means of the program of Statistica v 6.0 for Windows. Statistically significant difference between two groups were evaluated using the unpaired Student t-test ($p < 0.05$). The data are given as means ± SEM (standard error of mean).

COMPARE correlations

The graph of mean values for each of test compounds **5**, **7** and **8** was subsequently used to run the COMPARE algorithm from the Developmental Therapeutics Program (NCI) and calculate the correlation coefficient (https://dtp.cancer.gov/databases_tools/compare.htm) with respect to compounds from the standard agent database with a known mechanism of action. Pairwise correlation coefficients of greater than 0.5 were used as the cut-off for assessing whether two agents were likely to share a similar mechanism of action. Briefly, the GI_{50}, TGI and LC_{50} concentration vectors for the test compound were compared to those from the complete public database of standard agents.

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Нові фосфорильовані 5-(гідроксіалкіламіно)-1,3-оксазоли як потенційні протипухлинні агенти

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Резюме: Вісім нових фосфорильованих 5-(гідроксіалкіламіно)-1,3-оксазолів були розроблені та перевірені на їх здатність пригнічувати ріст ракових клітин. Ці сполуки оцінювали проти повних клітинних ліній пухлини людини NCI-60. Лише 3 сполуки показали протипухлинну активність в аналізі одноразової дози, які були взяті в аналізі п'яти доз. Сполуки **7** і **8** показали однакову середню антипроліферативну активність і цитотоксичність проти чутливих клітинних ліній загальної панелі. Однак сполука **8** виявила цитотоксичність для більшої кількості ліній, ніж **7**. За всіма параметрами ці сполуки були більш активними, ніж сполуки 5. Сполуки **7** і **8** також показали високу та подібну антипроліферативну активність у діапазоні концентрацій GI50: 1-6 і TGI: 6-14 мкМ проти всіх субпанелей. Їх цитотоксичність була в діапазоні концентрацій 25-54 мкМ. Сполука **5** показала таку ж активність, за винятком субпанелі лейкемії, недрібноклітинного раку легенів і раку яєчників, проти яких їх активність була нижчою. При аналізі структури-активності виявилося, що серед фосфорильованих похідних оксазолу протипухлинну активність виявляють лише сполуки, які містять катіон трифенілфосфонію (ТФР+) у 4-му положенні оксазольного циклу. Крім того, заміна фенільного радикала у 2-му положенні оксазольного скелета на метильний радикал призводила до зникнення активності. Алгоритм COMPARE виявляє високу кореляцію антипроліферативної активності досліджуваних сполук з протипухлинними агентами філантозидом і хромоміцином А3 у векторі GI50 і помірну з філантозидом у векторі TGI. Мішенню всіх стандартних препаратів, які корелюють з цитотоксичністю досліджуваних сполук, за винятком дідемніну, є ДНК. На відміну від стандартних сполук, синтезовані активні сполуки несуть делокалізований TPP+, який доставляє їх переважно до мітохондрій завдяки значно більшому гіперполяризованому потенціалу мітохондріальної мембрани в ракових клітинах, ніж у нормальних. Тому їх протипухлинна активність, швидше за все, зумовлена порушенням структурно-функціонального стану останніх через втручання в їхній внутрішній білоксинтезуючий апарат мітохондрій. Отримані дані дозволяють розглядати 5-гідроксіалкіл-аміно-1,3-оксазоли, наповнені TFP+, як провідні сполуки для подальшого поглибленого вивчення та синтезу нових TPP+-вмісних похідних 1,3-оксазолу з протипухлинною активністю.

Ключові слова: фосфорильовані 5-гідроксіалкіламіно-1,3-оксазоли; синтез; протипухлинна активність; порівняння кореляцій.