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Synthesis and antiproliferative activity of novel α- and β-dialkoxyphosphoryl isothiocyanates

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ABSTRACT

A series of 15 mostly new dialkoxyphosphoryl alkyl and aralkyl isothiocyanates were synthesized using two alternative strategies, and their in vitro antiproliferative activity against several cancer cell lines (including drug resistant) is here demonstrated. The IC_{50} values measured for the new compounds are within the range of 6.3–21.5 μM, and they are quite similar to the activity of two best and most extensively investigated natural benzyl isothiocyanate (**A**) and phenethyl isothiocyanate (**B**). Preliminary studies utilizing the cell cycle and reduced glutathione level analysis performed on A549 lung cancer cell line using representative compounds revealed important differences in the mechanism of action possibly correlated with their chemical properties. Hydrophobic compounds react mainly with the cytosolic glutathione reduced leading to its depletion, causing an oxidative stress and cell cycle arrest in G0/G1 phase. On the other hand, hydrophilic compounds cause moderate cell cycle arrest and massive cell death associated with moderate reduced glutathione depletion. These suggest that significant changes in the chemical structure of isothiocyanates, which do not lead to the significant changes in antiproliferative activity, but simultaneously cause a differences in the mechanism of action are possible.

Plants have been extensively studied throughout history as a potential source of anticancer drugs, and currently these drugs and their semi-synthetic analogues are widely used. They are utilized as such or in combination with other anticancer chemotherapeutic drugs in the treatment of a variety of cancer diseases. The most popular include: camptothecin, taxol and its semi-synthetic derivatives, vinblastine, etoposide and homoharringtonine, just to name a few among several hundred on the market today and in clinical trials.[1] There is a growing evidence which reports that a diet rich in certain types of vegetables, especially in cruciferous ones, plays an important role in protecting against cancer.[2,3] The cruciferous family of vegetables includes broccoli, cauliflower, watercress, cabbage, mustard, wasabi and horseradish. The chemoprotective effects of those vegetables are attributed to isothiocyanates, which are stored in plants in the form of inactive glucosinolate precursors. The activity of β-thioglucosidase (myrosinase) is a requirement for the conversion of glucosinolates to active isothiocyanates. This enzyme is separated in plant cells, but it is released when cells undergo damage. Alternatively, this crucial hydrolysis can be mediated by the microflora in the human gastrointestinal tract.[2–5]

Several years ago, The New York University School of Medicine in collaboration with The National Cancer Institute (NCI) initiated clinical trials investigating phenethyl isothiocyanate (**B**) in the prevention of lung cancer in people who smoke. Some other compounds from this group are extensively tested towards their possible antiproliferative activity (e.g., benzyl isothiocyanate and sulforaphane (1-isothiocyanato-4-(methylsulfinyl)butane)). However, there are more than 120 types of different precursors to isothiocyanates. The side chain of glucosinolates/isothiocyanates originates from natural occurring amino acids, consequently they do not represent significant structural variations.[6] Such variations are required for the modification of pharmacokinetic properties and thus some of the groups start to synthesize analogues of natural isothiocyanates. A new series of synthetic indole ethyl isothiocyanates were reported and their activity closely matches natural isothiocyanates,[7] but synthetic norbornyl isothiocyanates are weaker than sulforaphane.[8] In contrast, the selenium analogues of natural isothiocyanates, isoselenocyanates, are much more active in inhibiting the growth of melanoma, glioblastoma,

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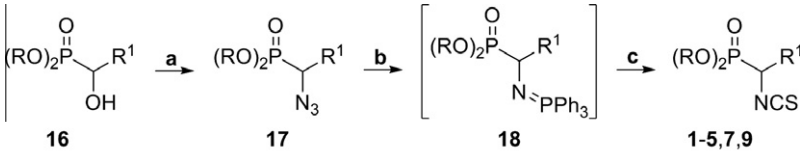
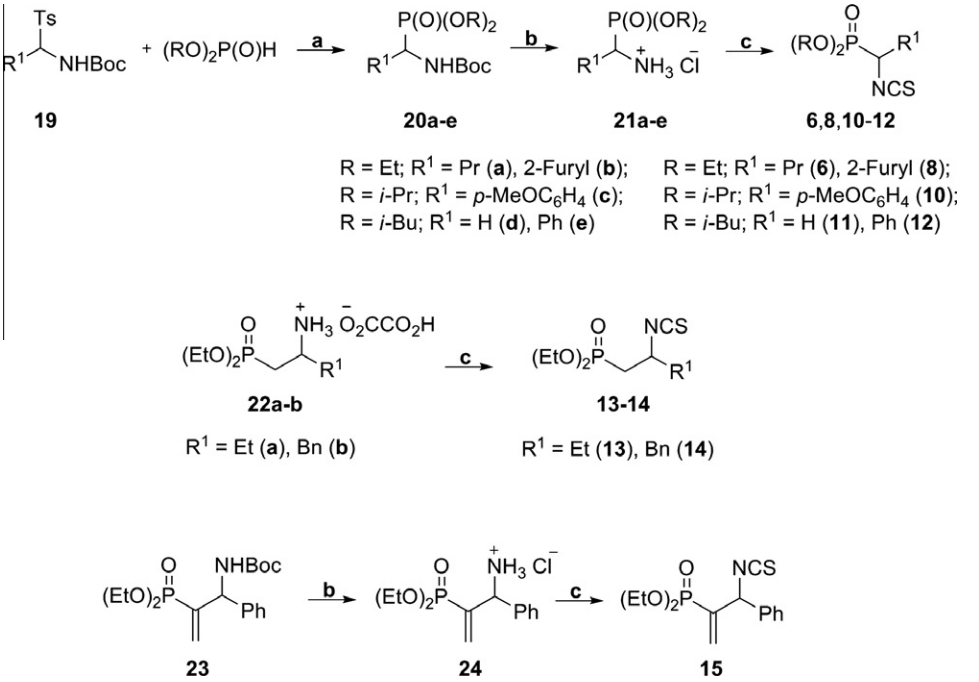


Scheme 1. Reagents and conditions: (a) Ph_3P/DEAD/HN_3/CH_2Cl_2, −10 °C to rt, 12 h; (b) Ph_3P (1.02 equiv)/toluene rt 3 h; (c) CS_2 (8 equiv), 90 °C, 6 h (for **1**, **2**,**4**, **5** and **9**), 14 h (for **3**) and 30 h (for **7**).



Scheme 2. Reagents and conditions: (a) NaH/THF rt, 4 h; (b) HCl/EtOH rt, 3 h; (c) DIPEA (4.8 equiv)/DMF/TBTU (1.2 equiv)/CS_2 (6.9 equiv), rt, 15 min.

firosarcoma, colon, breast and prostate cancer cells as compared to the corresponding natural isothiocyanates.[9]

It is questionable to which extent we can modify the isothiocyanate's structure and still preserve its anticancer properties. Comparison of the biological activity of some chemically diversed natural compounds (shown in Table 1, **A**–**D**) shows, that the presence of the isothiocyanate moiety is important but not sufficient for antiproliferative activity. It seems that, in addition to simple hydrophobic interactions, there is a requirement for the presence of other groups, for example, the phenyl ring, and this specific interaction could be replaced by the other functionality. In order to investigate this problem we have designed and synthesized a series of dialkoxyphosphoryl isothiocyanate derivatives. Our goal was to test a series of chemically diverse compounds containing dialkoxyphosphoryl moiety against a broad spectrum of human cancer cell lines. To our knowledge, this is the first Letter which describes the antiproliferative activity of such derivatives.

Two alternative strategies were applied in the synthesis of the target dialkyl 1-(isothiocyanato)- and 2-(isothiocyanato)alkylphosphonates (ITCP) **1–15**. In the first method (Scheme 1), introduced earlier in our laboratory,[10] easily available dialkyl 1-hydroxyalkylphosphonates[11] **16** were first converted into the appropriate azidoalkylphosphonates **17** via the Mitsunobu reaction[12] using triphenylphosphine (Ph_3P)/diethyl azodicarboxylate (DEAD) as a redox system and hydrazoic acid (HN_3) as a source of the azide anion.[13] Crude azides **17** were next converted via the Staudinger/aza-Wittig reaction[14] with Ph_3P into phosphazenes **18** followed by the reaction with carbon disulfide to give dialkyl 1-(isothiocyanato)alkylphosphonates **1–5**, **7**, **9** in 35–75% yields after flash chromatography.

In the second method, the desired dialkyl 1-(isothiocyanato) alkylphosphonates **6**, **8**, **10–12** and diethyl 2-(isothiocyanato) alkylphosphonates **13–15** were prepared from dialkyl 1-aminoalkylphosphonate hydrochlorides[15,16] **21a–e**, diethyl 2-aminoalkylphosphonate hemioxalates[17] **22a–b** and diethyl [1-(aminophenyl-methyl)-vinyl]phosphonate hydrochloride[18] **24**, respectively. The method established by Boas et al.[19] for the solid-phase synthesis of aliphatic isothiocyanates was adapted for the preparation of isothiocyanatophosphonates in a solution. The 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), used originally as a desulfurizating agent, was substituted for 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) in our modification. This resulted in a higher purity of the required ITCPs. As shown in Scheme 2, starting dialkyl 1-aminoalkylphosphonate hydrochlorides **21a–e** were prepared by the well established method[16] from dialkyl phosphonates and *N*-Boc α-aminoalkyl-*p*-tolylsulfones[16,20,21] **19**, followed by the

standard deprotection of the *N*-Boc group in **21a–e** with hydrogen chloride solution in ethanol. In turn, diethyl 2-aminoalkylphosphonate hemioxalates[17] **22a–b** were obtained from the easily available 2-hydroxyalkylphosphonates[17,22,23] by the well established method.

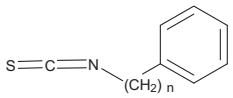
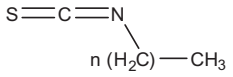
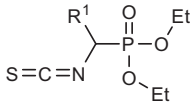
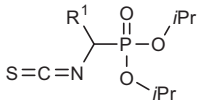
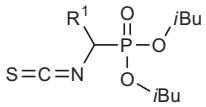
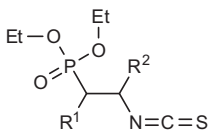
Straightforward conversion of aminophosphonate hydrochlorides **21a–e** and **24** and hemioxalates **22a–b** to dialkyl 1-(isothiocyanato)alkylphosphonates **6**, **8, 10–12** and diethyl 2-(isoth iocyanato)alkylphosphonates **13–15** was accomplished in the presence of diisopropylethylamine (DIPEA) by the reaction with carbon disulfide followed by desulfurization of such formed dithiocarbamates by TBTU (Scheme 2). The pure target isothiocyanates **6, 8**, **10–15** were isolated in 21–73% yields after flash chromatography or preparative TLC.

The in vitro antiproliferative activity of all of the fifteen synthesized compounds was evaluated using SRB assay against five human cancer cell lines—lung A549, breast T47D and MCF7, colon LoVo, as well as, its doxorubicin-resistant variant LoVo/DX. The 50% growth inhibitory concentration (IC_{50}) of the compounds on each cell line, including as a reference compounds four natural isothiocyanates (**A**–**D**), and widely used cytostatics are presented in Table 1. The activity of new isothiocyanates is quite similar to the activity of two best and most extensively investigated natural benzyl isothiocyanate (**A**) and phenethyl isothiocyanate (**B**) and for most of them, the IC_{50} values are within the range of 6.3–21.5 μM. These data provide evidence that the modification of the structure of natural isothiocyanates (**A** and **B**) (compounds **3**, **12** and **4,** respectively), by introducing an additional dialkoxyphosphoryl moiety or the larger dialkoxyphosphorylmethyl moiety (like in compounds **13** and **14**) does not change their antiproliferative properties compared to the parental natural isothiocyanates.

On the other hand, the modification of natural alkyl isothiocyanates (e.g., butyl isothiocyanate **C**) gives diethyl 1-(isothiocyanato)butylphosphonate (**6**) with 16 to 32 higher antiproliferative activity. In most cases, the tested compounds were as active as the cytostatics used as a control. However, it should be noted that the synthetic compounds, as well as, natural isothiocyanates are equally potent against non-multidrug-resistant and multidrug resistant cell lines. In fact, LoVo/DX doxorubicin-resistant cell line was the most sensitive for synthesized compounds, for example, the IC_{50} value for **9** was 2.5-fold lower compared to IC_{50} measured for LoVo cell line, and about twofold lower compared to values measured for the other cell lines used in the experiment. Therefore, we can assume that isothiocyanates with additional dialkoxyphosphoryl moiety possess the increased (compared to natural isothiocyanates **A**, **B**, **C** or **D)** ability to overcome multidrug resistance. None of the tested compounds show considerable selectivity against any of the cell line, but LoVo and LoVo/DX appeared to be the most sensitive ones.

This lack of diversity in antiproliferative activity is surprising, considering the significant differences in the chemical structure of the tested compounds. For a better understanding of this phenomenon, some basic physicochemical parameters for the natural and the synthetic isothiocyanates were calculated and are presented in Table 2.The *c* Log *P* and protein binding percentage are in good correlation. The caco-2 permeability values are the same for all of the synthetic compounds (about 21.7 ± 0.3 nm/s), which suggests a good membrane permeability and can partially explain

Table 1
Comparison of antiproliferative activity of natural isothiocyanates (**A**–**D**) and synthesized compounds (**1**–**15**) on several cancer cell lines in vitro

	Compound	R		Cancer cell line, IC_{50}[a] (μM)				
				Lung A549	Breast T47D	Breast MCF7	Colon LoVo	Colon LoVo/DX
	**A**	*n* = 1		20.5 ± 2	7.4 ± 0.8	18.9 ± 2.1	12.3 ± 1.5	10.5 ± 1.1
	B	*n* = 2		16.8 ± 1.1	9.7 ± 0.7	16.3 ± 1.4	13.5 ± 1.3	9.8 ± 0.4
	**C**	*n* = 3		413.8 ± 8	305.8 ± 22	389.8 ± 10	324.2 ± 36	231.4 ± 38
	D	*n* = 4		266.8 ± 32	174.4 ± 21	225.6 ± 30	41.5 ± 7	98.3 ± 23
	**1**	H		16.9 ± 1.2	14.7 ± 1.8	12.8 ± 1.3	9.7 ± 1.1	6.3 ± 1.2
	2	Me		16.6 ± 2.1	17.6 ± 1.6	16.0 ± 1.9	10.9 ± 2.2	8.8 ± 1.7
	3	Ph		13.5 ± 1.3	13.1 ± 1.4	13.1 ± 1.4	9.7 ± 1.7	7.4 ± 1.9
	4	Bn		14.5 ± 2.2	21.5 ± 3.7	18.1 ± 1.8	11.8 ± 0.9	12.9 ± 1.3
	5	*p*-$MeOC_6H_4$		15.1 ± 1.7	17.9 ± 1.9	13.5 ± 2.9	13.2 ± 2.1	10.5 ± 0.7
	6	Pr		16.8 ± 1.8	19.3 ± 3.1	12.2 ± 1.4	12.0 ± 1.9	8.8 ± 1.1
	7	1-Naphthyl		15.1 ± 1.1	11.9 ± 1.2	11.4 ± 1.9	9.6 ± 1.3	8.6 ± 1.7
	8	2-Furyl		16.2 ± 2.4	20.9 ± 1.4	11.8 ± 2.4	10.9 ± 2.2	11.3 ± 1.4
	**9**	H		15.3 ± 1.2	13.5 ± 2.3	13.8 ± 1.5	17.8 ± 1.6	6.9 ± 0.5
	10	*p*-$MeOC_6H_4$		15.7 ± 0.6	13.1 ± 2.8	11.4 ± 1.8	10.1 ± 1.1	11.7 ± 0.1
	**11**	H		10.9 ± 1.8	19.8 ± 3.3	14.7 ± 1.7	13.2 ± 1.6	16.6 ± 0.4
	12	Ph		17.2 ± 0.6	10.2 ± 0.9	11.9 ± 1.2	10.2 ± 0.2	11.9 ± 0.8
		R^1	R^2					
	**13**	H	Et	16.9 ± 0.7	17.7 ± 3.4	7.6 ± 1.9	8.0 ± 1.5	8.34 ± 0.9
	14	H	Bn	13.7 ± 0.6	13.4 ± 1.2	9.4 ± 0.9	9.8 ± 1.5	11.9 ± 1.3
	15	$=CH_2$	Ph	13.8 ± 0.2	12.9 ± 1.8	11.1 ± 1.5	8.6 ± 0.7	12.3 ± 1.6
Cytostatic[b]	–	–	–	13.9 ± 1.6	17.8 ± 1.4	15.2 ± 1.1	0.31 ± 0.1	12.2 ± 1.9

[a] Values are mean values ± SD measured using SRB assay from at least three independent experiments. Drug treatment for 72 h.
[b] In case of A549, T47 and MCF-7 cell lines cisplatin was used, in case of LoVo and LoVo/DX cell lines doxorubicin was used.

Table 2
Calculated in silico physicochemical parameters

Compound	*c* Log *P* [a]	Protein binding[b] (%)	Caco-2 permeability[b] (nm/s)
A	3.204	26	34.06
B	3.263	100	25.97
C	2.753	42	104.83
D	3.28	91	163.66
1	0.531	8	21.44
2	0.840	9	21.69
3	2.387	81	21.77
4	2.258	92	21.69
5	2.306	53	21.71
6	1.898	30	21.7
7	3.561	100	21.7
8	1.563	26	21.6
9	1.149	10	21.4
10	2.920	69	21.7
11	2.387	63	21.42
12	4.243	98	21.6
13	1.755	85	21.8
14	2.794	88	21.98
15	3.821	93	21.8

[a] Values were calculated using ChemBioDraw Ultra 11.0.
[b] Values were calculated using ADME prediction available on http://preadmet.bmdrc.org/.

Table 3
Cell cycle distribution percentage by flow cytometry[a]

	[G0/G1]/S ratio	%G0/G1	%S	%G2/M	%SubG0/G1
24 h					
Control	3.5	62 ± 2.4	18 ± 1.5	14.5 ± 1.2	4.5 ± 1.4
B	3.5	51 ± 1.1	14.5 ± 0.3	11 ± 1.5	22.5 ± 0.6
2	2.6	39 ± 0.2	15 ± 1.1	10 ± 0.8	34 ± 1.7
7	2.6	45 ± 1.2	16 ± 0.2	14 ± 0.9	24 ± 2.1
12	2.9	49 ± 1.2	18 ± 0.4	17.5 ± 0.4	14.5 ± 1.3
13	3.4	39 ± 1.8	12.5 ± 0.4	25.5 ± 1.7	20 ± 2.7
48 h					
Control	3.8	62 ± 1.2	17 ± 0.3	13.5 ± 0.7	6.5 ± 1.4
B	3.8	40.5 ± 2.8	10.5 ± 0.9	8 ± 0.7	39 ± 3
2	4	21 ± 0.6	5.5 ± 0.2	3.5 ± 0.7	69 ± 1.3
7	3	36 ± 0.6	11 ± 0.8	9.5 ± 1	42.5 ± 2.2
12	3.4	53.5 ± 3.1	17.5 ± 1.6	12 ± 0.2	14 ± 1.8
13	3.7	34 ± 0.3	9.5 ± 0.8	20.5 ± 1.4	34 ± 1.1
72 h					
Control	4.2	62 ± 3.3	16 ± 2.1	12.5 ± 1.7	7.5 ± 1.3
B	7.1	29 ± 8.1	4.5 ± 1.6	7.5 ± 2.4	54 ± 7.7
2	8.2	24.5 ± 0.6	3 ± 0.3	2.5 ± 0.4	68 ± 2.2
7	6.3	40 ± 3	6.5 ± 0.4	9.5 ± 0.3	42 ± 3.6
12	14.3	62.5 ± 2.3	3.5 ± 0.1	8.5 ± 0.7	22.5 ± 1.4
13	7.3	31 ± 2.3	4.5 ± 0.9	7.5 ± 0.6	55 ± 3.1

[a] Results represent mean values ± SD from three independent experiments. Compounds concentration—10 μM.

the lack of differences in activity. Surprisingly, there is no common relationship within *c* Log *P* values and antiproliferative activity. For example, compound **1** with *c* Log *P* only 0.52 is equally active as compound **12** for which *c* Log *P* value is 4.24. Conversely, the natural compounds **A** and **D** have almost identical hydrophobicity (*c* Log *P* = 3.2), but they are significantly different in their activity. It seems, that in addition, to the simple hydrophobic interactions there is a requirement for presence of the phenyl ring and this interaction could be replaced by the dialkoxyphosphoryl moiety (**1**, **9** and **11** vs **A** and **B**). This unexpected relation of the *c* Log *P* value compared to the observed activity could also be related to the currently unknown mechanism of action of the tested compounds.

Literature data suggest that isothiocyanates modifies covalently about 30 proteins, probably through a reaction with a low pK_a –SH moiety (S-thiocarbomoylation), including; tubulin,[24] phosphatase,[25] breast cancer resistance protein (ABCG-2)-mediated transport,[26] histone deacylase,[27] NF-κB,[28] and the most crucial for cancer development and survival the hypoxia inducible factor.[29] However, from the chemical point of view their main mechanism of action should be the thiocarbamoylation of cytosolic glutathione –SH group, resulting in the decrease of the redox status of the cells, which leads to cell cycle arrest and apoptosis.

In order to evaluate possible correlations between chemical structure of synthesized compounds and their mechanism of action, further experiments were performed utilizing diverse compounds **2**, **7**, **12** and **13** and **B** as the positive control. A549 cells were used and exposed to the chosen compounds for 24, 48 and 72 h, stained with propidium iodide, and analyzed using flow cytometry in order to determine the total population distribution in the cell cycle phases (Table 3). After 24 h of treatment a significant increase of the necrotic or the apoptotic cells population (subG0/G1) from 4.5% for the control to 34%, 20%, 24% and 14.5% for compounds **2**, **13**, **7** and **12**, respectively, was observed. No significant differences were observed in the number of cells in S phase (the greatest change for compound **13**—12.5% vs 18% for control). Only compound **13** led to an increase of cell population in the phase, G2/M from 14.5% for the control to 25.5%. After 48 h of treatment compound **2** caused a massive cell death (69% of the cells) and decreased the population of cells in G0/G1, S and G2/M phases when compared to the control. Compound **12** caused a slight increase in the number of cells in G0/G1, but the population of cells in subG0/G1 phase remained the same when compared to the data from the 24 h. After 72 h compound **2** did not lead to further changes in the cell cycle progress. Compound **12** treatment increased the percent of cells in subG0/G1 from 14.5% after 48 h to 22.5% after 72 h, lowered the number of cells in S and G2/M phase, respectively (especially in S phase from 16% for control to 4.5%), and did change the [G0/G1]/S ratio from 4.19 for the control to 14.3 which suggests a cell cycle arrest in the G0/G1 phase.

In the following experiments the level of reduced glutathione after the treatment with selected compounds was measured using the Ellman's assay.[30] Cells were exposed to the chosen compounds for 30 min, lysed and stained with 10 mM DTNB and the absorbance was read at 412 nm. Compound **12** lead to almost total depletion of GSH (Table 4, 8% compared to control). The electron withdrawing effect induced by the phenyl ring present in this compound is responsible for increased electrophilicity of carbon atom in isothiocyanate moiety which lead to a higher reactivity towards the cytosolic glutathione present in cells at high concentrations. Compounds **7** and **B** decreased the reduced glutathione level only by about 25%. Surprisingly, no correlation was found between *c* Log *P*, protein binding percentage and the glutathione depletion. However, there is a correlation between the glutathione depletion and [G0/G1]/S ratio change, which was found during cell cycle analysis. Compound **12** caused almost a complete depletion of GSH and a cell cycle arrest in G0/G1 phase, expressed by the high [G0/G1]/S ratio after 72 h treatment—14.3. On the contrary, compound **7** led to a minor cell cycle arrest compared to **12**; [G0/G1]/S ratio was 6.3 after 72 h, and the depletion effect was also low, but it increased subG0/G1 population two times more effectively than **12.** Compounds **2** and **13** led to a moderate GSH depletion (35% and 58%, respectively), and a moderate cell cycle arrest in

Table 4
Level of glutathione reduced [GSH][a]

Control	**B**	**2**	**7**	**12**	**13**
100% ± 4	75% ± 3	35% ± 2.5	76% ± 4	8% ± 1.5	58% ± 3.5

[a] Results are expressed as percentage of GSH level compared to control. Values are mean values ± SD from three independent Ellman's assay experiments. Compounds concentration—10 μM, drug treatment 30 min.

G0/G1 phase, but they caused the highest cell death level (68% and 55%, respectively). Treatment with the compound **7** (*c* Log *P* = 3.56) shows similar results in the phase cycle image and in a glutathione level depletion as in case of **B** (phenethyl isothiocyanate, *c* Log *P* = 3.26).

The cell cycle and the reduced glutathione depletion level analysis suggests a possible correlation between the structure and the mechanism of action of the synthesized compounds, thus hydrophilic compounds like **2** (*c* Log *P* = 0.84) exhibit their antiproliferative activity mainly causing cell death, when hydrophobic compounds like **12** (*c* Log *P* = 4.2) act through GSH depletion and cell cycle arrest in the G0/G1 phase, but do not cause a massive cell death. In fact, cell cycle arrest in G0/G1 is directly correlated with the glutathione depletion which leads to a decrease of the redox status of the cells. The redox model of cell proliferation (RMCP), as described by Hoffman[31] suggested that within normal cells the cell cycle is stopped by a redox switch (towards the more oxidative environment) in G0/G1 phase. The above described redox switch helps to regulate proliferation via phosphorylation and dephosphorylation of redox-sensitive regulatory proteins such as the retinoblastoma protein (pRb). Due to metabolic and chromosomal changes the cancer cells do not possess such checkpoint, and the redox switch does not occur, pRb cannot be dephosphorylated and the cells can proliferate easily. However, such redox switch can be induced artificially, for example, by isothiocyanates, and can lead to the cell cycle inhibition. Such phenomena can also be observed for some dialkoxyphosphoryl isothiocyanates, especially for compound **12**.

In conclusion, the antiproliferative activity of α- and β-dialkoxyphosphoryl alkyl and aralkyl isothiocyanates, against a variety of cancer cell lines, was evaluated. All of the synthesized isothiocyanates showed a very good antiproliferative activity in vitro, comparable to the most active of natural isothiocyanates and the commonly used cytostatics—cisplatin and doxorubicin, however no significant differences in the overall IC_{50} values were observed. The cell cycle and GSH level analysis highlights significant differences in the mechanism of action of the tested compounds, which could be correlated with their chemical structure. Hydrophobic compounds react mainly with the cytosolic reduced glutathione leading to its depletion, causing an oxidative stress which leads to cell cycle arrest in G0/G1 phase rather than cell death. On the other hand, hydrophilic compounds cause a moderate cell cycle arrest and a massive cell death associated with the moderate reduced glutathione depletion. These data suggest that significant changes in the chemical structure of isothiocyanates, which do not lead to significant changes in the antiproliferative activity, but simultaneously cause a differences in the mechanism of action are possible. Clearly more of the synthetic isothiocyanate derivatives are needed to resolve the previously mentioned issue of structure-mechanism of action relationship. A new derivatives reported here (except **1**, **9** and **11**) possess asymmetric carbons and were tested as racemic mixtures. It is interesting to investigate, if there are some differences between the enantiomers and such approach could be helpful in the search of an appropriate mechanism of action for isothiocyanates. The synthesis of such derivatives is in progress.

Supplementary data

Supplementary data (synthesis and experimental procedures along with the full characterization for the compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.113.

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