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Convenient syntheses of novel 1-isothiocyano-alkylphosphonate diphenyl ester derivatives with potential biological activity

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ABSTRACT

Herein, we describe a convenient method for the syntheses of novel 1-isothiocyano-alkylphosphonate diaryl ester derivatives and their antiproliferative activity. The syntheses are based on dithiocarbamates obtained in situ with the use of carbon disulfide under basic conditions, and their desulfurization using several different reagents, of which hydrogen peroxide proved to be the most efficient. The compounds synthesized demonstrated high antiproliferative activity against several cancer cell lines in vitro, and also showed some activity as serine protease inhibitors.

The isothiocyanate moiety is a useful functional group in organic syntheses, acting as a strong electrophile with the carbon atom as an electrophilic center. It has proved to be useful in a wide variety of synthetic applications, for example in pseudo-peptide thiourea syntheses[1] and in heterocyclic chemistry.[2] Isothiocyanates (ITC) are also found in plants in large amounts and have a high degree of structural diversity.[3] In numerous in vitro and in vivo studies these naturally occurring isothiocyanates (such as benzyl isothiocyanate and allyl isothiocyanate) have shown high antiproliferative and anticancer activity (see the review by Zhang et al.[4]), and have negligible toxicity. Such advantages make them very promising natural compounds for cancer treatment. A key phase responsible for the above activities is fast cellular accumulation via passive diffusion followed by the cellular glutathione-isothiocyanate reaction and/or protein modification resulting in cell cycle arrest and apoptosis. As a result, the intracellular concentration of isothiocyanates can reach high levels (e.g., after 30 min of treatment, intracellular concentrations can reach 9.4 mM in Hepa 1c1c7 cells[5]). We believe that such a phenomenon could be utilized in order to transport additional biologically active moieties present in the same structure. In such an approach, even moieties with lower biological activity could be useful due to their accumulation inside the cells, which is especially crucial for compounds such as enzyme inhibitors for example.

The mechanism of action of natural and synthetic isothiocyanates is not clear. These electrophilic species could simply deplete cellular glutathione and/or react with some of the very important cellular–SH proteins. In the first case, there is no clear structure activity–relationship, therefore any new synthetic isothiocyanate could provide additional evidence for such a mechanism of action. Here, we describe a series of new isothiocyanates containing a phosphonate diaryl ester moiety. We have found that these compounds are potent antiproliferative agents, but also, like other diaryl phosphonate esters,[6] they are able to inhibit irreversibly serine proteases.

Among several methods for the syntheses of isothiocyanates, those based on primary amine conversion using thiophosgene, di-2-pyridyl thionocarbonate, or carbon disulfide are among the most extensively used. Since thiophosgene is extremely toxic and di-2-pyridyl thionocarbonate is relatively expensive, we chose carbon disulfide to convert primary amine hydrobromides into the corresponding isothiocyanates. In this method, the amine moiety is first converted into the corresponding dithiocarbamate by reaction with carbon disulfide in the presence of a base in a water miscible organic solvent, followed by subsequent desulfurization with an appropriate reagent. Three different desulfurization agents were tested: *O*-benzotriazole-*N*,*N*,*N*1,*N*1-tetramethyluronium

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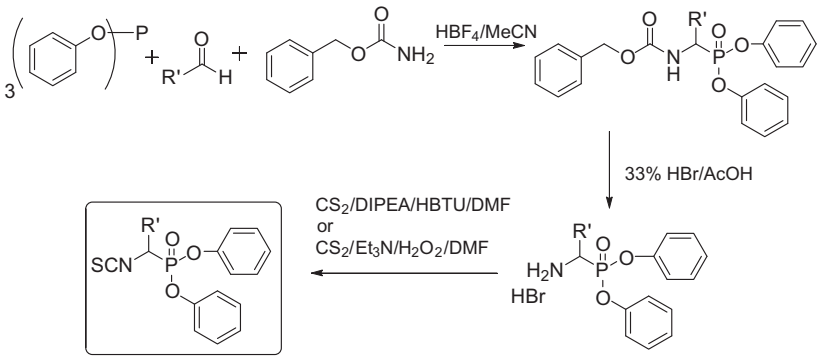
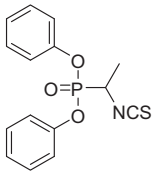
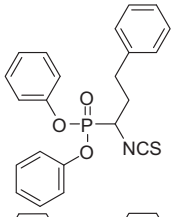
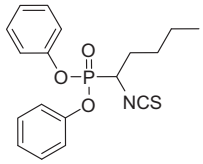
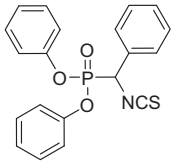
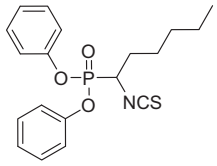
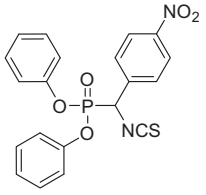
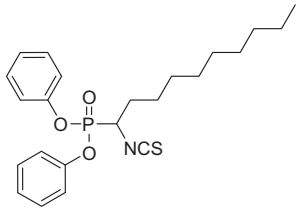
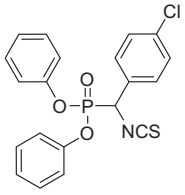
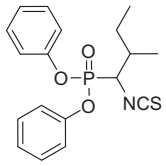
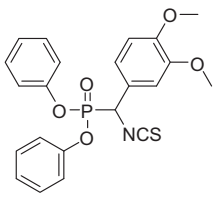


Scheme 1.

Table 1
Chemical structure and yield of the obtained isothiocyanates

Product		Yield (%) HBTU/H_2O_2	Product		Yield (%) HBTU/H_2O_2
	**1**	78/84		**6**	79/75
	**2**	72/86		**7**	57/55
	**3**	84/92		**8**	67/64
	**4**	90/93		**9**	86/77
	**5**	77/90		**10**	77/85

hexafluorophosphate (HBTU), tosyl chloride (TsCl), and hydrogen peroxide (H_2O_2). In all the methods, 1-amine-alkylphosphonate diaryl ester hydrobromides, obtained using a modified α-amidoalkylation reaction,[6] followed by a standard Cbz-deprotection, were used as the substrates (see Scheme 1).

The method based on HBTU as the desulfurization agent, as previously reported by Boas[7] and used in the solid-phase syntheses of thioureas, provided us with the target compounds in moderate to high yields and with satisfactory purity. However, several side products were always present along with the target compound, thus purification via silica gel column chromatography was necessary. The use of tosyl chloride as the desulfurization agent did not improve the yields or the purities of the final isothiocyanates, therefore this reagent was not tested further. The conversion of the amine hydrobromide into isothiocyanate with the use of hydrogen peroxide, previously applied to hydroxyalkyl isothiocyanate synthesis,[8] allowed us to obtain the target compounds in high yields, and in higher purities in comparison to the HBTU-based

Table 2
The antiproliferative activity of the synthesized compounds against several cancer cell lines in vitro

ITC	IC_{50} [μM] ± SD			
	LoVo	LoVoDX	A549	MCF7
1	10 ± 1	11 ± 1	36 ± 2	29 ± 1
2	10 ± 1	10 ± 1	47 ± 2	33 ± 1
3	12 ± 1	54 ± 2	68 ± 2	60 ± 2
4	70 ± 1	71 ± 3	86 ± 2	79 ± 1
5	16 ± 2	17 ± 1	34 ± 2	30 ± 1
6	13 ± 1	21 ± 1	17 ± 1	34 ± 2
7	13 ± 1	27 ± 1	18 ± 1	33 ± 2
8	69 ± 2	68 ± 2	66 ± 3	80 ± 3
9	19 ± 1	12 ± 1	59 ± 2	40 ± 2
10	7 ± 1	8 ± 1	31 ± 2	20 ± 1

method. Nevertheless, only compounds **1-3** and **6** (see Table 1) were obtained as satisfactorily pure products, and for all the other compounds the crude purity was 65–80%. However, the impurities could easily be removed using flash chromatography. It should be noted that in this method the temperature of the reaction had to be kept in the range of 0–15 °C in order to maintain the yield and purity at high levels. Allowing the reaction mixture to warm up during H_2O_2 addition led to the formation of several side products, and in most cases, separation of the target compound was difficult.

In both of the methods described, the base employed was triethylamine (Et_3N); *N,N*-diisopropylethylamine (DIPEA) can be used with no detrimental influence on the yield and purity. Considering the fact that the isothiocyanate moiety readily reacts with a primary amine under basic conditions resulting in thiourea derivative formation, the pH of the reaction should be kept below 8 to avoid such a side reaction, and/or an excess of CS_2 should be added in order to ensure full conversion of the amine substrate into the dithiocarbamate salt. For most of the compounds the best yields and purities were achieved when 4–6 equiv of DIPEA and 10–15 equiv of CS_2 were used along with 1.5 equiv of HBTU as the desulfurization agent; 1.1-1.5 equiv of Et_3N, and 10–15 equiv of CS_2 when 3–4 equiv of hydrogen peroxide are used. However, in the case of compounds **7** and **8**, the conversions, regardless of the method used, decreased significantly. For example, from 57% for **7** and 67% for **8** to 11% and 9%, respectively, when four molar equivalents of DIPEA were used in the HBTU-based method. The most probable explanation for this is the instability of the corresponding hydrobromides under basic conditions. For the other compounds described in this Letter, such decomposition was not observed.

All of the compounds synthesized were tested for their antiproliferative activity against breast (MCF7), lung (A549), and both doxorubicin-sensitive (LoVo) and doxorubicin-resistant (LoVoDX) colon cancer cell lines. The results are presented in Table 2. For the most active compounds, (**1**, **2** and **10**) the antiproliferative effects were comparable to those of the naturally occurring isothiocyanates.[9] Clearly, more compounds need to be synthesized and examined in order to evaluate the possible correlation between the chemical structure of the obtained isothiocyanates, their biological activity and their potential as anticancer agents. Additionally, the inhibitory potency of the synthesized compounds against human neutrophil elastase and chymotrypsin was measured using fluorescent substrates. Some of them inhibited these serine proteases with k_2/K_{inact} in the range of 80–870 M^{-1} s^{-1}. However, due to the presence of two electrophilic centers, the mechanism of inhibition is not clear and investigations of this issue are underway.

In summary, a rapid and convenient method for the syntheses of novel 1-isothiocyano-alkylphosphonate diaryl esters, as building blocks in organic chemistry and as anticancer agents, has been described. Their in vitro antiproliferative activity against cancer cells is in the range of natural isothiocyanates despite the significant differences in their structures. This seems to suggest that depletion of glutathione is the main mechanism for the antiproliferative potential of isothiocyanates, when –NCS reactivity with small molecules (such as GSH, glutathione sulfide) is not influenced by the size and shape of other regions of molecules (excluding electron-withdrawing effects). Additionally, the obtained compounds possess serine protease inhibitory activity. In our opinion such compounds are a good starting point to develop a new class of biologically active agents as potential drugs or as tools in cancer studies.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.08.037. These data include MOL files and InChiKeys of the most important compounds described in this article.

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