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In vitro radical scavenging and cytotoxic activities of novel hybrid selenocarbamates

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ABSTRACT

Novel selenocyanate and diselenide derivatives containing a carbamate moiety were synthesised and evaluated in vitro to determine their cytotoxic and radical scavenging properties. Cytotoxic activity was tested against a panel of human cell lines including CCRF-CEM (lymphoblastic leukaemia), HT-29 (colon carcinoma), HTB-54 (lung carcinoma), PC-3 (prostate carcinoma), MCF-7 (breast adenocarcinoma), 184B5 (non-malignant, mammary gland derived) and BEAS-2B (non-malignant, derived from bronchial epithelium). Most of the compounds displayed high antiproliferative activity with GI_{50} values below 10 μM in MCF-7, CCRF-CEM and PC-3 cells. Radical scavenging properties of the new selenocompounds were confirmed testing their ability to scavenge DPPH and ABTS radicals. Based on the activity of selenium-based glutathione peroxidases (GPxs), compounds **1a**, **2e** and **2h** were further screened for their capacity to reduce hydrogen peroxide under thiol presence. Results suggest that compound **1a** mimics GPxs activity. Cytotoxic parameters, radical scavenging activity and ADME profile point to **1a** as promising drug candidate.

1. Introduction

Due to the lack of selectivity and increased resistance to anticancer drugs, there is an urgent need to develop new and safer drugs for effective treatment of cancer.[1,2] In this field selenium derivatives have caught great attention due to their wide variety of biological activities.[3–6] Although it is noticeable that the chemical form of selenium is a determinant for its biological activity,[7,8] the mechanisms that drive the selenium-anticancer action are not fully understood. Nevertheless, since oxidative stress is a hallmark of a variety of chronic human diseases commonly attributed to radical scavenging and enzymatic decomposition of oxygen metabolites, the antioxidant properties of organoselenium compounds have been extensively investigated.[9–11] Interestingly, recent data from our laboratory have demonstrated that some organoselenium derivatives presented both antiproliferative and antioxidant activities.[12]

Among the different synthetic selenium compounds that have been developed, the chemical forms selenocyanate and diselenide have received significant attention.[13–19] Selenocyanate function has proven effectiveness in the prevention and treatment of a variety of cancers, both in vitro and in vivo. Among their actions inhibition of m-TOR signalling,[13] tubulin polymerization[14] and nitric oxide levels,[15] and modulation of antioxidative enzymes such as glutathione peroxidase, glutathione-S-transferase, thioredoxin reductase, superoxide reductase and catalase[14–17] have all been described. Diselenide compounds could play a beneficial role in oxidative diseases acting as antioxidants that can protect against cancer lesions. In fact, a series of substituted diaryl diselenides behaved as cytotoxic and apoptotic inducers in HT-29 cells,[18] while other diselenide derivatives have shown ability to inhibit histone deacetylase and PI3K/Akt signalling in WM35 and UACC 903 melanoma cells.[19] In addition, diphenyl diselenide is able to protect both MCF-7 breast cancer cells from oxidative DNA damage induced by tamoxifen[20] and J774 macrophage-like cells from oxidized LDL-mediated effects.[21] Moreover, some diselenides have been designed as potential therapeutic agents mimicking the peroxidase activity of selenium based glutathione peroxidases.[22]

Taking into account our previous experience in selenocyanate and diselenide chemistry[23–25] and the fact that we have recently reported some carbamates as potential antiproliferative drugs,[26] we decided to further extend our research by synthesising thirty new hybrid compounds in which selenocyanate or diselenide and carbamate moieties were combined. In these compounds the carbamate function, a group commonly found in antitumour active

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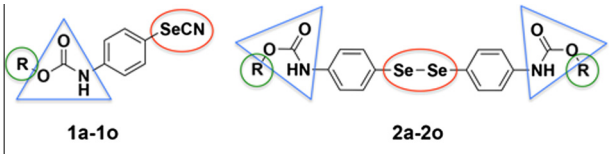
compounds[27–32] acts as a link between the central and ending scaffolds, thus enabling their chemical accessibility and increasing polarity in this area as compared to our previously described structures.[23,33–35] Besides, this group might facilitate the hydrolysis of the compounds towards anionic species that could act as prodrugs (Fig. 1).

To explore the influence on the activity of the molecular modifications of the ending cores we decided to compare the effect of different aryl and short-chain alkyl substituents commonly used in medicinal chemistry. During the last years it has been established that short-chain and medium-chain length fatty acids, as well as long-chain polyunsaturated fatty acids can be used as chemotherapeutic agents for cancer treatment. For instance, while monounsaturated fatty acids from sixteen to twenty two carbon atoms have shown to inhibit proliferation in breast cancer cells,[36] lauric acid derivatives induced apoptosis in colon cancer,[37] and the carbamate scaffold linked to tert-butyl[38] or butyl chains resulted active against lung and colon cancer.[39] Additionally, it has been described that some fatty acids can act synergistically with classic chemotherapeutic agents.[40] Keeping this in mind, here we analyse how short chain fatty acids (4–7 atoms in length) linked to the carbamate scaffold affect activity.

To study the impact on the activity of different aromatic rings, and considering the synthetic accessibility, we also synthesised a diverse pool of phenyl derivatives either with one electron-donating or one electron-withdrawing substituent that carried or not a methylene group as a separating moiety, increasing both bond flexibility and adaptability of bulky derivatives. In some cases the aromatic ring was either a polycyclic system such as Fmoc, that has been used previously as an antitumour agent conjugated to peptides,[41] or a heteroaromatic unit such as the benzo[*b*]thiophene-1,1-dioxide.[42]



1a-1o 2a-2o

Compounds	R
1a/2a	Ethyl
1b/2b	Propyl
1c/2c	Butyl
1d/2d	Hexyl
1e/2e	Isobutyl
1f/2f	Allyl
1g/2g	Phenyl
1h/2h	4-Methoxyphenyl
1i/2i	4-Methylphenyl
1j/2j	4-Chlorophenyl
1k/2k	4-Fluorophenyl
1l/2l	Benzyl
1m/2m	4-Nitrobenzyl
1n/2n	(fluoren-9-yl)methyl (Fmoc)
1o/2o	1,1-dioxobenzo[*b*]thiophen-2-yl

Figure 1. Structure of new synthesised hybrids.

Inspired by aforementioned above, we herein report the design, synthesis, and in vitro cytotoxic and cytostatic activities, as well as the selectivity, against a panel of human cell lines for 30 new selenocarbamate derivatives. The capability of the title compounds to interact with the stable radical 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$), their efficiency to scavenge 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{+\cdot}$) and their ability to mimic glutathione peroxidase activity was also examined.

2. Results and discussion

2.1. Chemistry

General procedure for selenocyanate (compounds **1a**–**o**) and diselenide (compounds **2a**–**o**) derivatives synthesis is illustrated in Scheme 1. The first step for **1a**–**o** preparation was the generation of 4-aminophenylselenocyanate according to a previous reported procedure.[31] Treatment of 4-aminophenylselenocyanate with the appropriated chloroformates, synthesised according to standard procedures[43,44] or purchased commercially, for 24 h at room temperature in dry chloroform and 1:1 molar ratio, gave **1a**–**o** in a 4–29% yield. Formation of carbamates was confirmed following the wavenumber position in infrared spectroscopy peaks: carbonyl group showed up around 1780 cm^{-1} in the starting chloroformates whereas the carbonyl in the carbamates arose around 1706–1735 cm^{-1}. In addition, the triple bond of SeCN group was detected between 2141 and 2159 cm^{-1}. Crude reaction products were purified by recrystallization and/or washed with organic solvents.

The synthetic route for **2a**–**o** was based on the reaction between 4,4-diaminodiphenyldiselenide with the corresponding chloroformates in a 1:2 molar ratio, in dry chloroform at room temperature for 24 h. The starting intermediate 4,4-diaminodiphenyldiselenide was prepared according to the method described by Banks et al.[45] **2a**–**o** diselenide derivatives, whose carbamate carbonyl group was detected at 1684–1726 cm^{-1}, were recovered at variable yields (2–63%) after washing with ethyl ether or hexane.

Chemical structures for every synthesised compound were confirmed by ^{1}H NMR, ^{13}C NMR, MS spectral and elemental analysis. NMR spectral data of the compounds showed all proton and carbon signals at their expected chemical shift values. No sophisticated instrumentation, conditions or reagents were required for the transformations that took place under mild conditions.

2.2. Biological evaluation

2.2.1. Cytotoxicity

Synthesised compounds were screened for their cytotoxic and antiproliferative activities against a panel of human tumour cell lines as a representative selection of solid, liquid and hormone-dependent tumours, including lung carcinoma (HTB-54), colon carcinoma (HT-29), lymphocytic leukaemia (CCRF-CEM), breast adenocarcinoma (MCF-7) and prostate adenocarcinoma (PC-3). As a guide with regard to selectivity all of the compounds were further examined for toxicity in two cell lines derived from non-malignant cells: 184B5, a cell line established from normal breast tissue,[46] and BEAS-2B, a cell line derived from non-malignant bronchial epithelium cells.[47] Cytotoxicity assays were based on the reactivity of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] as previously described.[48] The cytotoxic effect of each substance was tested at five different concentrations between 0.01 and 100 μM. GI_{50}, that is, the concentration that reduces by 50% the growth of treated cells with respect to untreated controls, TGI, the concentration that completely inhibits cell growth, and LC_{50}, the concentration that kills 50% of the cells