European Journal of Medicinal Chemistry 83 (2014) 674–684

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

# Synthesis and antiproliferative activity of novel methylselenocarbamates

Beatriz Romano [a], María Font [a], Ignacio Encío [b], Juan Antonio Palop [a, *], Carmen Sanmartín [a]

[a] Departamento de Química Orgánica y Farmacéutica, University of Navarra, Irunlarrea 1, E-31008 Pamplona, Spain
[b] Departamento de Ciencias de la Salud, Universidad Pública de Navarra, Avda. Barañain s/n, E-31008 Pamplona, Spain

ARTICLE INFO

*Article history:*
Received 2 May 2014
Received in revised form
11 June 2014
Accepted 30 June 2014
Available online 1 July 2014

*Keywords:*
Carbamate
Methylseleno
Cytotoxicity
Apoptosis
Cell cycle arrest

ABSTRACT

A series of new aliphatic, aromatic and heteroaromatic carbamate derivatives containing a methylseleno moiety were synthesized and evaluated *in vitro* for their cytotoxic activity against a panel of human cell lines including CCRF-CEM (lymphoblastic leukaemia), K-562 (lymphocytic 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 are highly cytotoxic with $GI_{50}$ values below 10 μM in every tested tumour cell line. Based on its cytotoxic parameters, selectivity index and ADME profile, the biological activity of compound **2**, the propyl derivative, was further analysed in CCRF-CEM and HTB-54 cells. Results showed that this compound is able to induce apoptosis in a time- and dose-dependent manner. Involvement of caspases in cell death induction by **2** was detected. Besides, compound **2** was also able to induce cell cycle arrest at $G_0/G_1$ in CCRF-CEM cells and at $G_2$/M in HTB-54 cells.



## 1. Introduction

Cancer is a serious clinical problem: it affects millions of patients worldwide, reduces life quality and is one of the leading causes of death [1]. At present, there are many antitumour drugs available for clinical use. Despite this fact, treatment of cancer still presents many obstacles and both, lack of selectivity with the consequent side-effects and occurrence of intrinsic or acquired resistance of tumours to chemotherapy point out the necessity to develop new anticancer therapies [2]. In this context, selenium compounds have attracted considerable attention during the last decade since they have been shown to inhibit tumour development and growth in a variety of cancer [3–9]. In particular, converging lines of evidence support the hypothesis that methylselenol is a key intermediate metabolite for effective cancer prevention and treatment by methyl-selenium compounds [10–13].

Over the last years we have been involved in the development, design and synthesis of structurally modified selenium derivatives, some of which exhibited significant cytotoxic and antiproliferative activity through induction of apoptosis, cell cycle arrest and/or modulation of different kinases [14–23]. Among these compounds, we described some methylseleno derivatives whose cytotoxic activity depends on the release of methylselenol [10,14,18,22,24]. Upon this, and considering the chemical structural patterns previously described by us, we designed a general structure for the new compounds presented here. This structure (Fig. 1) keeps a central scaffold of *Se*-methylselenourea, a group whose effectiveness to release methylselenol has been proven. Since carbamates are considered privileged scaffolds in cancer therapy [25–28], keeping a general standard of molecular symmetry [18] and based on a hybrid design approach, here, we try the presence of two carbamate functions attached to this group. Carbamate functions have several roles: first, they act as a link between central and ending scaffolds enabling their chemical accessibility; second, they slightly increase polarity in this area as compared to the structures that we have previously described [14,16,18,21,23]; and third, they theoretically enable the hydrolysis of the compounds towards anionic species that could act as prodrugs. Besides, to explore the influence of the ending pieces of the symmetric molecule on its activity we introduced either an aliphatic or an aromatic structure in these zones.

The use of fatty acids as adjuvants in cancer treatment is well established. *In vitro* studies have displayed the capacity of these

* Corresponding author. Department of Organic and Pharmaceutical Chemistry, University of Navarra, Irunlarrea 1, E-31008 Pamplona, Spain.
*E-mail address:* jpalop@unav.es (J.A. Palop).

http://dx.doi.org/10.1016/j.ejmech.2014.06.076
0223-5234/

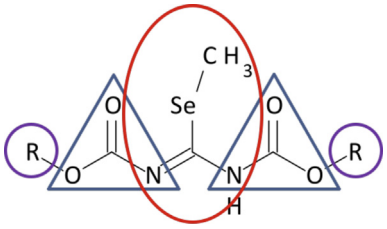



**Fig. 1.** General structure of new pharmacophoric hybrids.

acids, categorized by the number of carbon atoms in the aliphatic chain, to increase the activity of standard chemotherapeutic agents in a range of cell lines [29–33]. Thus, to juggle with the carbamate group and to imitate the structure of short chain fatty acids, we performed a bioisosteric replacement of a methylene group by an oxygen atom of ether, which causes a slight increase in polarity while keeping a similar bond angle and chain flexibility. Following this approach, here we tested short chain fatty acids (4–7 atoms in length). On the other hand, we selected a series of aromatic rings [14] bearing either one electron-donating group or one electron-withdrawing group to test aromatic endings. These rings were either directly linked to the carbamate, or separated from it by a methylene group. In two cases, the ring was a polycyclic system such as Fmoc [34] or a heteroaromatic unit such as the benzo[*b*]thiophene-1,1-dioxide [35].

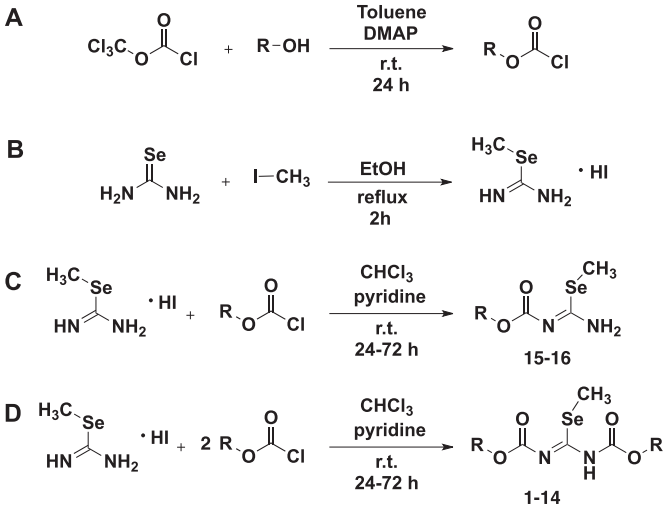
Additionally, the cytotoxic activity of every synthesised compound was tested against a panel of six human tumour cell lines as a representative selection of solid, liquid and hormone-dependent tumours, including breast adenocarcinoma (MCF-7), colon carcinoma (HT-29), lymphocytic leukaemia (K-562), lymphoblastic leukaemia (CCRF-CEM), prostate carcinoma (PC-3) and lung carcinoma (HTB-54). As a guide with regard to selectivity, two cell lines derived from non-malignant cells, one from mammary gland (184B5) and the other from bronchial epithelium (BEAS-2B), were also tested. Moreover, to further analyse the mechanism of action of the new compounds, the ability to induce apoptosis and cell cycle arrest of derivative **2**, one of the most active and selective compounds, was also tested.

## 2. Results and discussion

### 2.1. Chemistry

The general synthetic route used to obtain the desired bisimidoselenocarbamates was straightforward and it is outlined in Scheme 1. The synthesis was performed from *Se*-methylselenourea hydroiodide, obtained by a method previously developed in our laboratory [18], and the corresponding chloroformates, synthesized according to standard procedures [36,37] or purchased commercially. The reaction was carried out stirring at room temperature for 24–72 h in a 1:2 molar ratio using dried chloroform and in the presence of pyridine. When necessary, dimethylaminopyridine (DMAP) was also included as a basic catalyst. After isolation and purification of the compounds, this procedure gave yields within the range of 5–78%.

Since chloroformates bearing different groups on the aryl ring behave differently, this reaction outcome might be due to the influence of these substituents. For instance, replacement of electron-donating groups on the aromatic ring (**8** and **9**) by electron-withdrawing groups (**10** and **11**) offered a noticeable decrease in yield from 41% to 5%. Moreover, all our attempts to generate the corresponding 4-nitrophenylbiscarbamate were unsuccessful since the reaction of 4-nitrophenylchloroformate with




**Scheme 1.** Synthesis of compounds **1**–**16**. (**A**) Preparation of the corresponding chloroformate. (**B**) Preparation of *Se*-methylselenourea. (**C**) Synthesis of compounds **15**–**16**. (**D**) Synthesis of compounds **1**–**14**.

methylimidoselenocarbamate hydroiodide in different conditions (temperature, solvents, catalyst) always yielded 4-nitrophenol. Hence, to test the influence of the nitro group with regards biological activity, we decided to replace it with a nitrobenzyl group (**13**).

The same traditional synthetic procedure was used for the preparation of the bis derivative of 1,1-dioxobenzo[*b*]thiophenyl. Unfortunately, this procedure failed to afford the expected product and the monomer derivative (**15**) was isolated instead (78% yield). This prompted us to seek alternative routes to prepare the corresponding 1,1-dioxobenzo[*b*]thiophenylbiscarbamate. To our surprise, modification of the reaction conditions (temperature, solvents, molar ratio, catalyst…) resulted in decomposition of both, reagents and monomer yielding complex reaction mixtures.

Despite some of the derivatives (**7**, **10** and **13**) were isolated in a poor yield because their purification was troublesome, all newly synthesized compounds are pure and stable and their structures were confirmed by spectroscopic (IR, $^{1}$H NMR and $^{13}$C NMR), mass spectrometry (MS) and elemental analysis.

### 2.2. Biological evaluation

#### 2.2.1. Cytotoxicity

Every synthesised compound was screened for its cytotoxic and antiproliferative activity against CCRF-CEM, K-562, MCF-7, HT-29, PC-3 and HTB-54 cells. Cytotoxicity assays were performed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] method as previously described [38]. The cytotoxic effect of each substance was tested at five different concentrations between 0.01 and 100 μM. Obtained results are shown in Table 1 and are expressed as $GI_{50}$, i.e. the concentration that reduces by 50% the growth of treated cells compared to untreated controls, TGI, the concentration that completely inhibits cell growth, and $LC_{50}$, the concentration that kills 50% of the cells.

As shown in Table 1, CCRF-CEM and HTB-54 cells were generally more sensitive and K-562 more resistant to the cytotoxic effect. In fact, most of the target compounds were active as cytostatic agents against HTB-54, PC-3, CCRF-CEM and MCF-7 cells, with $GI_{50}$ values below 5.5 μM. Moreover, compounds **1**, **2**, **3**, **6**, **7**, **10**, **12** and **15** in HTB-54 cells, **1**, **5**, **7**, and **10** in PC-3 cells, **1**, **2**, **5**, **7**, **9**, **10**, **11**, **12**, **13**, **14**, **15** and **16** in CCRF-CEM cells, **1**, **7**, **10** and **11** in HT-29 cells and **15** in