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Research paper

Chalcogen containing heterocyclic scaffolds: New hybrids with antitumoral activity

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ABSTRACT

In this work, 27 novel hybrid derivatives containing diverse substituents with chalcogen atoms (selenium or sulfur) and several active heterocyclic scaffolds have been synthesized. Compounds were tested against two human cancer cells lines (MCF7 and PC-3) and a normal human mammary epithelial cell line (184B5) in order to determine their activity and selectivity against malignant cells. Ten compounds showed GI_{50} values below 10 μM in at least one of the cancer cell lines and six of them exhibited a selectivity index higher than 9. In general, selenium-containing compounds were more active than their corresponding sulfur analogs but we found some thiocyanate derivatives with comparable or higher activity and selectivity. Among the different substituents, the seleno- and thio-cyanate groups showed the most promising results. On the basis of their potent activity and high selectivity index, compounds **7e** and **8f** (containing a thiocyanate and a selenocyanate group, respectively) were selected for further biological evaluation. Both the compounds induced caspase-dependent cell death and cell cycle arrest in G_2/M phase. In addition, these compounds do not violate any of the Lipinski's Rule of Five and thus possess good potential to become drugs, compound **7e** being particularly promising.

1. Introduction

Cancer is one of the leading causes of death worldwide. Its prevalence is expected to continue growing due to the spread and aging of the population, as well as an increase of established risk factors such as smoking, overweight, poor diet and physical inactivity [1]. One of the main modes of treatment for cancer is chemotherapy. However, the efficacy of current chemotherapeutic agents is limited by the development of tumor resistance and the appearance of undesired side effects [2,3]. Therefore, the design of more effective and safer cancer therapeutic agents is still necessary.

In the last decade, selenium-containing compounds have generated a growing interest due to their efficacy and selectivity against cancer cells [4,5]. The biological activity of selenium (Se) compounds is highly dependent on their chemical form [4,6]. Among the different synthetic Se compounds that have been developed, the chemical forms methylseleno, selenocyanate and selenol have received significant attention. The methylseleno entity and its precursors have been recognized as a key intermediate for cancer treatment [7,8]. On the other hand, different compounds that incorporate the selenocyanate group, such as 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) and diphenylmethyl selenocyanate (DMSE), have shown marked antitumoral effects alone or in combination with other drugs [9,10]. In addition, our group has demonstrated the promising effects for these entities in previous works [11–13].

As a novel approach, herein we compare the effects of these three different Se-containing substituents incorporated into different heterocyclic scaffolds which have been traditionally used as anticancer agents: benzo[*d*]thiazole [14,15], pyrimidine [16,17], acridine [18,19], quinoline [20,21], phthalimide [22,23], benzodioxole [24,25], isoxazole [26,27] and phenylpyrrole [28]. The selected heterocycles are structurally diverse enough in order to confer different volume, rigidity, and electronic and lipophilic

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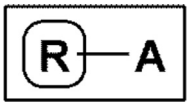
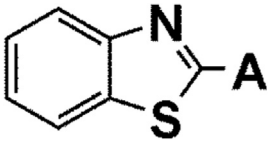
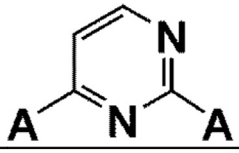
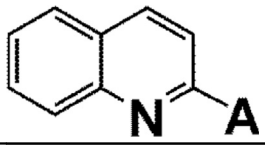
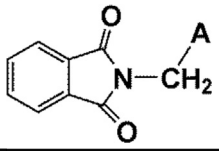
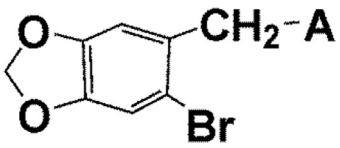
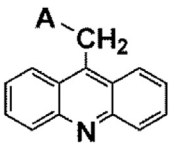
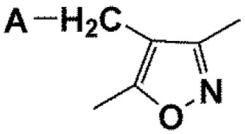
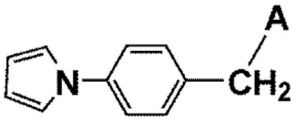
characteristics to the target molecules. Furthermore, alkyl and aryl halides were selected in order to study the effects of a methylene group between the ring and the substituents.

Generally, several studies have reported an enhancement of the cytotoxic activity when sulfur (S) was substituted by Se [29–32], although it is not always the case [11,33]. Furthermore, in spite of the fact that these two elements belong to the same group of the periodic table, they show different biological behavior. Therefore, to compare the anti-cancer properties of the above mentioned Se compounds with their S isosteres, some of the corresponding S analogs were also synthesized and screened.

Table 1
Structure of the novel Se- and S-containing compounds.



Ref.	R	A
1a		-SH
1b		-SeH
1c		-S-CH_3
1d		-Se-CH_3
1e		-SCN
2a		-SH
2b		-SeH
2c		-S-CH_3
2e		-SCN[a]
3a		-SH
3c		-S-CH_3
3d		-Se-CH_3
4a		-SH
4b		-SeH
4e		-SCN
4f		-SeCN
5a		-SH
5b		-SeH
5e		-SCN
5f		-SeCN
6b		-SeH
6e		-SCN
6f		-SeCN
7e		-SCN
7f		-SeCN
8e		-SCN
8f		-SeCN

[a] Monosubstituted in position **2**.

This study reports the synthesis of 27 novel Se-containing compounds as well as some of their corresponding S analogs. Compounds were tested against two human cancer cell lines (MCF7 and PC-3) and a normal cell line (184B5) to study both their activity and selectivity. Moreover, to further analyze the mechanism of action, the ability of the lead compounds to induce cell cycle arrest and cell death was also investigated.

2. Results and discussion

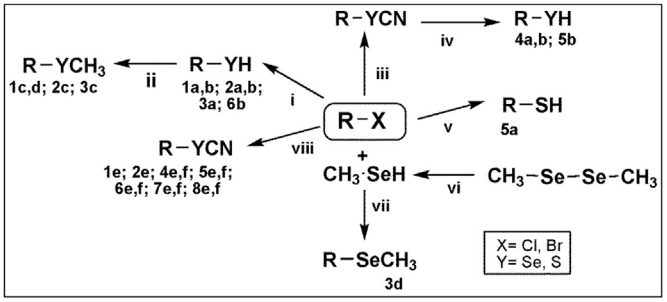
2.1. Chemistry

Twenty seven novel compounds with different Se- and S-containing substituents have been synthesized. Their structures are summarized in Table 1. The compounds were prepared according to previously published procedures [11,34–37] with some modifications, as shown in Scheme 1, starting from the commercially available aryl or alkyl halides. The purity of all the products was determined by thin layer chromatography (TLC) and elemental analysis, and was 99% or higher. The structures were confirmed by IR, ^{1}H and ^{13}C NMR, and mass spectrometry (MS).

The introduction of the selenol and thiol groups in aryl halides was carried out by treating the halides with seleno- or thio-urea in a 1:1.1 (aryl halide:seleno-/thio-urea) molar ratio at reflux using absolute ethanol or dry methanol as solvents [11]. The same method was employed for the synthesis of compound **6b** from corresponding alkyl halide. However, a similar reaction strategy failed to yield thiols **4a** and **5a** and selenols **4b** and **5b** from corresponding alkyl halides. This was in accordance with our previous report [30], where the reaction between an alkyl halide and seleno- or thio-urea resulted in the formation of the corresponding carbamimidoselenoate or carbamimidothioate instead of the selenol or thiol.

We propose a two-step mechanism for the synthesis of these compounds (Fig. 1). The first step could be the nucleophilic attack by the lone pair electrons of the Se atom over the carbon bearing the halogen following a classical S_N2 mechanism. The product thus formed could suffer hydrolysis or alcoholysis owing to the slightly acidic conditions generated by the release of the hydrohalic acid after the S_N2 reaction. This last step yields the thiol or selenol derivatives and would occur only under certain reaction conditions, times and depending on the electrophilic character of the carbon atom on Se–C(NH_2)=NH. In summary, the hydrolysis/alcoholysis of the intermediate carbamimido could explain the chemical behavior observed for these compounds.

In general, aryl halides give the hydrolysis/alcoholysis under the



Scheme 1. General procedure of synthesis. Reagents and conditions: (i) Seleno-/thio-urea, absolute ethanol or dry methanol, 0.5–15 h, reflux; (ii) 1. NaOH, DMSO, 1 h, r.t. 2. CH_3I, 4 h, r.t; (iii) KSeCN/KSCN, acetone, 2 h, reflux; (iv) 1. $LiAlH_4$, dry THF, 2–4 h, r.t., dark, N_2. 2. Succinic acid, 0.5–1.5 h, r.t or 0 °C; (v) 1. KSAc, dry *N*,*N*-DMF, 16 h, r.t. 2. NaOH, methanol, 4 h, r.t.; (vi) $NaBH_4$, absolute ethanol, r.t, N_2, 30 min (vii) Absolute ethanol, 5 h, reflux; (viii) KSeCN/KSCN, acetone, 2–24 h, reflux.