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Phenolic thio- and selenosemicarbazones as multi-target drugs

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ABSTRACT

A series of isosteric phenolic thio- and selenosemicarbazones have been obtained by condensation of naturally-occurring phenolic aldehydes and thio(seleno)semicarbazides. Title compounds were designed as potential multi-target drugs, and a series of structure-activity relationships could be established upon their *in vitro* assays: antioxidant activity, α-glucosidase inhibition and antiproliferative activity against six human tumor cell lines: A549 (non-small cell lung), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast) and WiDr (colon). For the antiradical activity, selenium atom and 2 or 3 phenolic hydroxyl groups proved to be essential motifs; remarkably, the compound with the most potent activity, with a trihydroxyphenyl scaffold (EC_{50} = 4.87 ± 1.57 μM) was found to be stronger than natural hydroxytyrosol, a potent antioxidant present in olive oil (EC_{50} = 13.80 ± 1.41 μM). Furthermore, one of the thiosemicarbazones was found to be a strong non-competitive inhibitor of α-glucosidase (K_i = 9.6 ± 1.6 μM), with an 8-fold increase in activity compared to acarbose (K_i = 77.9 ± 11.4 μM), marketed for the treatment of type-2 diabetes. Most of the synthesized compounds also exhibited relevant antiproliferative activities; in particular, seleno derivatives showed GI_{50} values lower than 6.0 μM for all the tested cell lines; *N*-naphthyl mono- and dihydroxylated derivatives behaved as more potent antiproliferative agents than 5-fluorouracil or cisplatin.

1. Introduction

Schiff bases are attractive and versatile scaffolds with relevant applications in several areas, including asymmetric catalysis [1], chemosensors [2], photochemical switches [3], live cell imaging [4], or pharmacology [5], among others. Remarkably, triapine™, a simple 3-aminopyridinyl-based thiosemicarbazone has progressed through Phase I and II clinical trials and is currently a promising pharmaceutical drug exhibiting potent anticancer activity against an ample panel of human tumors [6]. Furthermore, potent *in vitro* antiparasitic activity against the protozoan *Toxoplasma gondii* [7], the agent causing toxoplasmosis disease, and *Trypanosoma cruzi* [8], provoking the Chagas disease (or American trypanosomiasis) has also been reported for thiosemicarbazones. Some other relevant biological properties associated to these Schiff bases include antimicrobial activity [9] and inhibition of xanthine oxidase [10] involved in the catabolism of purines to give uric acid.

Herein we report the preparation of a series of phenolic thiosemicarbazones and their selenium isosters; we have analyzed the influence of the number and position of the phenolic hydroxyl groups, the nature of the chalcogen atom, and the type of *N*-aryl substituent on the antioxidant and antiproliferative activities, and on glycosidase inhibition, with the aim of developing potential multi-target drugs. Such approach is the basis of an emerging and promising area within Medicinal Chemistry research coined as polypharmacology [11], targeted at the treatment of complex multifactorial diseases, which require the simultaneous modulation of a network of targets.

2. Results and discussion

2.1. Chemistry

Taking into consideration that Shiff bases are endowed with a

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; DPPH, 2,2-diphenyl-1-picrylhydrazyl, free radical; EC_{50}, half maximal Effective Concentration; FTC, Ferric Thio Cyanate method; GI_{50}, Growth Inhibition of 50%; NCI, National Cancer Institute; ROS, Reactive Oxygen Species; TBARS, thiobarbituric acid reactive substances.

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series of biological activities mainly due to their redox and chelating capacities, we envisioned the possibility of combining a phenolic scaffold with thio- or selenosemicarbazone moieties (Fig. 1). The strong antioxidant properties associated to both phenolic and organoselenium compounds are widely known [12]. Furthermore, these families of compounds have also attracted a considerable pharmaceutical interest [13]. Therefore, an enhancement of the bio-activities in the target compounds might take place *via* synergic effects when both functionalities are simultaneously present.

The synthetic route for accessing phenolic thio- and selenosemicarbazones is illustrated in Scheme 1. Thus, derivatives **10**–**20** were obtained from moderate to almost quantitative yields (28–93%) by acid-promoted condensation of a series of naturally-occurring phenolic aldehydes (*p*-hydroxybenzaldehyde **1**, 3,4-dihydroxybenzaldehyde or protocatechuic aldehyde **2**, 2,4,5-trihydroxybenzaldehyde **3**, and 3,4,5-trihydroxybenzaldehyde **4**) with commercially-available thiosemicarbazide **5** or its selenium analogs **8** (R = Ph) [14] and **9** (R = α-naphthyl). Selenosemicarbazides were, in turn, obtained from the corresponding aryl isoselenocyanates **6**–**7** [15] by addition of hydrazine monohydrate. ^{13}C NMR spectrum of the hitherto unknown 4-(α-naphthyl)selenosemicarbazide **9** showed the presence of the characteristic selenoxo group, with a resonance of 178.4 ppm.

Compounds **9**–**20** were isolated by column chromatography, or directly by recrystallization from the crude reaction media to give highly crystalline colored solids; spectral data fully confirmed the structures of these Schiff bases. Thus, ^{1}H NMR spectra of **10**–**20** depicted the presence of the imine-type proton, located at 7.92–8.33 ppm, and NH protons involved in strong hydrogen bonding with the solvent (DMSO-d_6), as deduced from their remarkable deshielding (11.55–12.08 ppm). The resonance of both, the thioxo and selenoxo groups in ^{13}C NMR showed no appreciable differences (roughly 173–175 ppm).

2.2. Antioxidant activity

Compounds **9**–**20** were tested as antioxidant agents against *Reactive Oxygen Species* (ROS), a series of highly reactive intermediates produced in mitochondrial respiratory processes, or induced by exogenous agents, and whose accumulation in tissues is responsible for the oxidative stress, a cellular state with deleterious effects on virtually all biomolecules [16]. Oxidative stress has been demonstrated to play a predominant role in chronic inflammation and in a plethora of degenerative processes, like cell aging, cardiac damage, Parkinson's and Alzheimer's diseases [17]. Furthermore, increased generation of ROS coming from mitochondrial dysfunction also constitutes a common and decisive feature in the initial stages of many types of cancer [18].

In particular, derivatives **9**–**20** were evaluated as scavengers of free radicals, H_2O_2 and alkyl peroxides, and the results are depicted in Table 1.

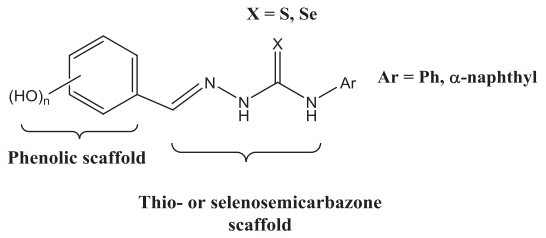

Fig. 1. General structure of the targeted phenolic thio(seleno)semicarbazones.

2.2.1. Antiradical activity

The antiradical activity of title compounds was evaluated using the DPPH method [19]. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free-radical with a strong absorbance at 515 nm; in the presence of an antioxidant, the absorbance is consequently reduced upon formation of the corresponding hydrazine by H-donation. The antiradical activity was estimated by calculating EC_{50}, that is, the concentration of antioxidant required to scavenge 50% of the initial free radical. Data depicted in Table 1 suggest that an increase in the number of phenolic hydroxyl groups leads to a significant improvement of the antiradical activity, especially when changing from one to two hydroxyls. Concerning the starting aldehydes, *p*-hydroxybenzaldehyde **1** shows no antiradical activity at a concentration of 250 μM, whereas 3,4-dihydroxybenzaldehyde **2** exhibits an EC_{50} value of 6.57 ± 0.79 μM. The same tendency was especially remarkable for thiosemicarbazones **10**–**11** (90.10 ± 13.46 *vs.* 9.27 ± 1.87 μM) and selenosemicarbazones **14**–**15** (27.80 ± 1.61 μM *vs.* 8.77 ± 2.23 μM).

The much lower EC_{50} values obtained for monohydroxylated derivatives **10**, **14** and **17** in comparison with *p*-hydroxybenzaldehyde is a clear evidence of the positive effect of incorporating a thioxo or selenoxo group for the antiradical activity of the tested compounds. In this context, selenosemicarbazide **9**, lacking phenolic hydroxyl groups, exhibited an EC_{50} value of 6.65 ± 0.67 μM, virtually the same as that obtained for the most potent compound, the trihydroxy selenosemicarbazone **16** (4.87 ± 1.57 μM), and the most potent aldehyde **3** (5.97 ± 0.41 μM). Moreover, derivatives **9**, **11**, **13**, **15**, **16**, **19**, and **20** turned out to be stronger antiradical agents (up to a three-fold increase) than hydroxytyrosol (2-(3′,4′-dihydroxyphenyl)ethanol), a natural polyphenol especially abundant in olive tree [20], and partially responsible for the nutraceutical properties of extra virgin olive oil [21]. This simple phenolic derivative, a potent antioxidant, is usually taken as a reference compound (EC_{50} = 13.80 ± 1.41 μM) when measuring the antiradical properties of naturally-occurring and synthetic polyphenols.

2.2.2. Hydrogen peroxide scavenging

Hydrogen peroxide is an important ROS in the cellular metabolic pathway, being especially abundant in mitochondria, in a process regulated by nitric oxide [22]. The capacity of scavenging H_2O_2 by the prepared compounds was evaluated following the methodology reported by Bahorun et al. [23]. In this assay, phenol red indicator is subjected to oxidation with H_2O_2 in a process promoted by horseradish peroxidase to furnish a chromophore with a strong absorption at 610 nm. Reduction of the absorbance in the presence of an antioxidant agent is directly correlated with the scavenging activity of such compound.

Thio- and seleno derivatives **9**–**20**, together with starting aldehydes **1**–**4** were tested at a final concentration of 100 μM; the percentage of inhibition at this concentration is depicted in Table 1. In this assay, the most potent derivatives turned out to be the thiosemicarbazides **10**–**13**, with scavenging activities of roughly 80%. Furthermore, the thioxo and selenoxo groups proved to be again important motifs; thus, *p*-hydroxybenzaldehyde **1** lacked anti-H_2O_2 activity at the tested concentration, whereas thio- and seleno-derivatives **10**, **14** and **17**, also with one single phenolic hydroxyl moiety, showed 53.1–79.5% scavenging activity at the same concentration. In general, the seleno-isosters presented reduced activity, in particular the *N*-naphthyl derivatives, the latter case possibly due to steric hindrance.

2.2.3. Lipid peroxidation inhibition (Ferric Thiocyanate Method, FTC)

High concentration of ROS provokes degradation of lipid bilayers, particularly at the doubly allylic positions of