European Journal of Medicinal Chemistry 94 (2015) 63–72

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Phenolic thio- and selenosemicarbazones as multi-target drugs

Verónica Calcatierra [a], Óscar López [a, *], José G. Fernández-Bolaños [a], Gabriela B. Plata [b], José M. Padrón [b]

[a] Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Apartado 1203, E-41071 Seville, Spain
[b] BioLab, Instituto Universitario de Bio-Orgánica "Antonio González" (IUBO-AG), Centro de Investigaciones Biomédicas de Canarias (CIBICAN), Universidad de La Laguna, c/ Astrofísico Francisco Sánchez 2, E-38206 La Laguna, Spain

ARTICLE INFO

Article history:
Received 1 October 2014
Received in revised form
13 January 2015
Accepted 19 February 2015
Available online 21 February 2015

Keywords:
Phenolic compounds
Thio(seleno)semicarbazones
Antioxidants
ROS scavengers
Glycosidase inhibitors
Antiproliferative agents

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.

* Corresponding author.
E-mail address: osc-lopez@us.es (Ó. López).

http://dx.doi.org/10.1016/j.ejmech.2015.02.037
0223-5234/

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.

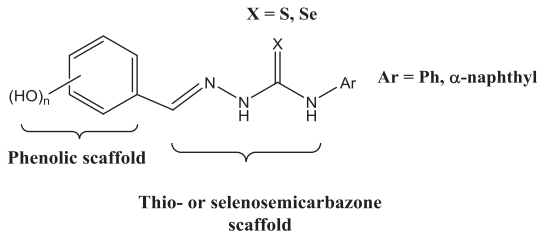


Fig. 1. General structure of the targeted phenolic thio(seleno)semicarbazones.

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.

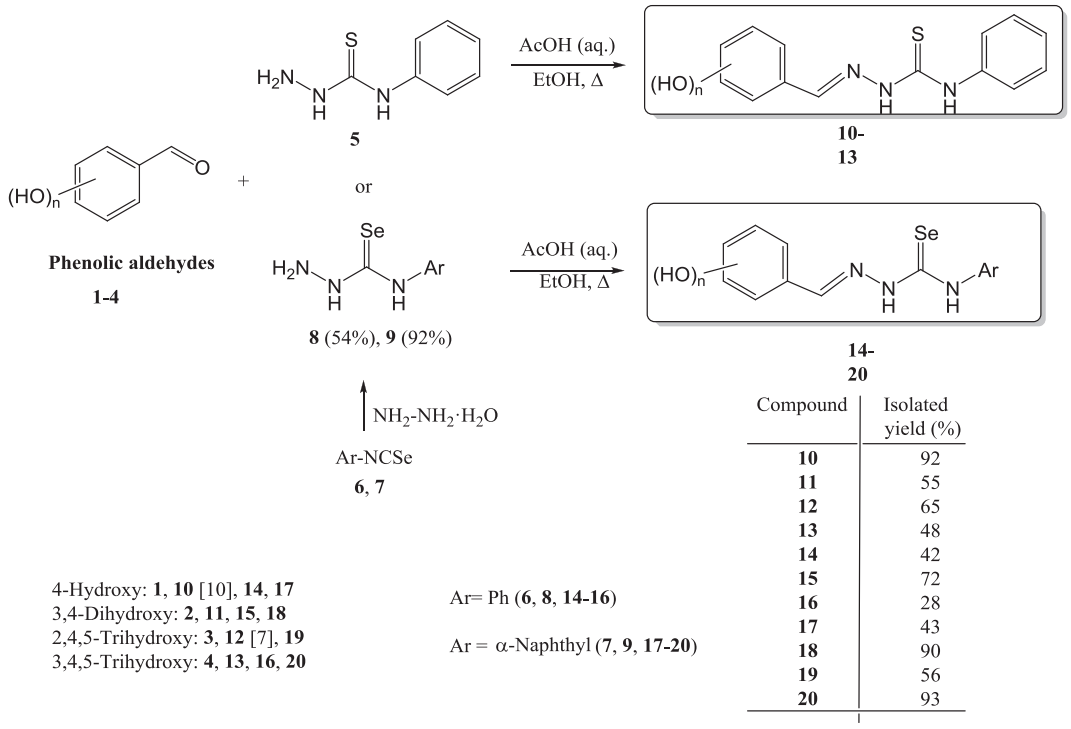
2.2.2. Hydrogen peroxide scavenging

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)

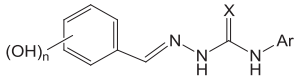
High concentration of ROS provokes degradation of lipid bilayers, particularly at the doubly allylic positions of



Compound	Isolated yield (%)
10	92
11	55
12	65
13	48
14	42
15	72
16	28
17	43
18	90
19	56
20	93

Scheme 1. Preparation of phenolic thio- and selenosemicarbazones **10**–**20**.

Table 1
Antioxidant activity of compounds **9**–**20** compared to starting aldehydes **1**–**4**.



		Compound				Assay	
		$(OH)_n$	X	Ar	DPPH method (EC_{50}, μM)	%H_2O_2 scavenging[a]	%Lipid peroxidation inhibition[b]
Aldehydes	**1**	4-Hydroxy	–	–	>250	–[c]	–[c]
	2	3,4-Dihydroxy	–	–	6.57 ± 0.79	78.3 ± 1.1	35.7 ± 2.2
	3	2,4,5-Trihydroxy	–	–	5.97 ± 0.41	80.3 ± 1.2	56.1 ± 2.0
	4	3,4,5-Trihydroxy	–	–	15.35 ± 0.70	79.1 ± 1.1	–[c]
	9	–	–	–	6.65 ± 0.67	56.9 ± 0.6	21.9 ± 2.0
Thiosemicarbazones	**10**	4-Hydroxy	S	Ph	90.10 ± 13.46	79.5 ± 0.2	5.5 ± 2.4
	11	3,4-Dihydroxy	S	Ph	9.27 ± 1.87	79.3 ± 0.9	72.0 ± 1.9
	12	2,4,5-Trihydroxy	S	Ph	13.9 ± 1.23	–[c]	56.8 ± 2.2
	13	3,4,5-Trihydroxy	S	Ph	7.0 ± 0.65	80.0 ± 0.9	59.9 ± 1.5
Selenosemicarbazones	**14**	4-Hydroxy	Se	Ph	27.80 ± 1.61	72.5 ± 0.2	31.0 ± 2.5
	15	3,4-Dihydroxy	Se	Ph	8.77 ± 2.23	69.0 ± 0.6	66.5 ± 1.6
	16	3,4,5-Trihydroxy	Se	Ph	4.87 ± 1.57	64.0 ± 0.8	65.7 ± 1.5
	17	4-Hydroxy	Se	$C_{10}H_7$	21.67 ± 2.31	53.1 ± 0.7	23.3 ± 2.2
	18	3,4-Dihydroxy	Se	$C_{10}H_7$	18.17 ± 2.19	46.3 ± 0.7	72.0 ± 1.6
	19	2,4,5-Trihydroxy	Se	$C_{10}H_7$	10.70 ± 0.73	4.5 ± 0.4	43.0 ± 5.0
	20	3,4,5-Trihydroxy	Se	$C_{10}H_7$	8.87 ± 1.47	38.1 ± 1.1	24.3 ± 3.1

[a] At 100 μM.
[b] At 0.74 mM.
[c] No activity at the tested concentration.

polyunsaturated fatty acid residues, what therefore affects the physical properties of cell membranes, like permeability [24]. Furthermore, lipid peroxidation is also quite a frequent process in breast cancer, due to the abundant adipose tissue surrounding the mammary gland, and the pro-oxidant environment generated [18].

The lipid peroxidation process was herein reproduced following the procedure reported by Olszewska et al. [25], using linoleic acid as the model compound and AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride), a water-soluble azo compound, as a thermal free radical initiator to induce the lipid degradation. The subsequent alkyl peroxides generated oxidize Fe(II) to Fe(III) which in turn furnishes a brick-red color complex with thiocyanate anion, detectable by UV–Vis spectroscopy.

All the compounds (tested at a final concentration of 0.74 mM) exhibited inhibition of lipid peroxidation, except for 4-hydroxybenzaldehyde **1**, and 3,4,5-trihydroxybenzaldehyde **4**,

(Table 1). Especially remarkable are di- and trihydroxy thio(seleno) semicarbazones **11** (72.0 ± 6.25%), **15** (66.4 ± 6.1%), **16** (65.6 ± 5.9%) and **18** (71.9 ± 5.6%), which behaved as more potent alkyl peroxide scavengers than the corresponding initial aldehydes (35.8 ± 2.2% and 56.1 ± 5.1%). These results show that not only the catechol framework is important for this activity, but the thioxo and selenoxo groups present in the corresponding Schiff base architectures provoke a substantial enhancement (up to a two-fold increase).

2.3. Pro-oxidant activity

It has been reported that some polyphenols exert both, antioxidant and pro-oxidant activities on a concentration-dependent manner [26]. Although it is usually considered as a deleterious effect, the pro-oxidant properties of a substance, that is, the generation of ROS, might be useful, for instance, in certain anticancer therapies [27]. In this context, the potential pro-oxidant activities of thio- and selenosemicarbazones has been analyzed, and the results are depicted in Table 2.

For this purpose, a Fenton reaction model [28] containing $FeCl_3$–EDTA–H_2O_2 was used; under these conditions, hydroxyl radicals are generated, provoking the degradation of 2-deoxyribose. The extent of such degradation was evaluated by treatment with thiobarbituric acid to give the so called thiobarbituric acid reactive substances (TBARS) that can be measured spectrophotometrically at 532 nm.

The solvent used for each sample was DMSO; as it can be observed, the control experiment shows a low absorbance value, what can be attributed to the scavenging properties of DMSO [29]. This value is increased when in the control experiment, DMSO is replaced by water.

For all the tested compounds, selenosemicarbazide **9**, thiosemicarbazones **10**, **12**, and selenosemicarbazones **14**, **15**, **18** and **20**, a second measurement was accomplished without deoxyribose, in order to take into consideration possible interactions of the above compounds with TBARS. For all of them, a clear pro-oxidant effect was observed at high concentrations (0.83 mM), showing absorbance values higher than the control experiment. This effect was particularly significant for monohydroxylated thio- and selenosemicarbazones **10** and **14**, 3,4-dihydroxy *N*-phenyl selenosemicarbazone **15** and 3,4,5-trihydroxy-*N*-naphthyl selenosemicarbazone **20**. Moreover, for derivative **18**, the same study was conducted on a range of concentrations from 0.83 to 0.083 mM; as it can be observed, the pro-oxidant activity varies in concentration-dependent mode, being more pronounced at higher concentrations.

Table 2
Pro-oxidant activity of thio- and selenosemicarbazones[a].

Compound	Abs	Abs' (without deoxyribose)	$\mathbf{A}_{sample} - \mathbf{A'}_{sample}$
Control (DMSO)	0.038	–	–
Control (H_2O)	0.155	–	–
9	0.231	0.167	0.064
10	0.108	0.014	0.094
12	0.230	0.163	0.067
14	0.161	0.074	0.087
15	0.191	0.094	0.097
18	0.180	0.101	0.079
	0.111[b]	0.044	0.067
	0.060[c]	0.019	0.041
	0.039[d]	0.002	0.037
20	0.262	0.165	0.097

[a] At 0.83 mM.
[b] At 0.42 mM.
[c] At 0.17 mM.
[d] At 0.083 mM.

2.4. α-Glucosidase inhibition

Carbohydrate-processing enzymes are ubiquitous biocatalysts involved in a series of relevant processes like sugar, glycolipid and glycoprotein metabolic routes, molecular recognition, or cell wall formation [30]; one of the most widely-studied families of this kind of enzymes are glycosidases, that catalyze the hydrolysis and formation of glycosidic bonds. Defective glycosidases are responsible for the development of certain metabolic disorders and diseases, like lysosomal storage disorders (e.g. Gaucher or Fabry diseases) or type-2 diabetes [26]. Nowadays it is widely accepted that tumor cells are featured with aberrant glycosidase activities [31] and therefore the corresponding glycosidases can be a promising anticancer therapeutic target.

Stimulated by the fact that some plant extracts have shown hypoglycemic properties, attributed to the α-glucosidase inhibition exerted by their phenolic constituents [32], we decided to test compounds **9**–**20**, and aldehydes **1**–**4** against α-glucosidase from baker's yeast (Table 3). For this purpose, *p*-nitrophenyl-α-D-glucopyranoside was used as the model substrate; the formation of the corresponding *p*-nitrophenolate in a buffered medium (pH 6.8) was followed at 400 nm. Initially, title compounds were tested at a final concentration of 500 μM using a substrate concentration (250 μM) equal to the calculated value of the enzymatic Michaelis–Menten constant (K_M, 0.25 ± 0.03 mM), in order to calculate the percentage of inhibition. Under these conditions, phenolic aldehydes did not show any inhibition, and selenosemicarbazide **9**, only 14% of inhibition. Nevertheless, at the same concentration, all thio- and selenosemicarbazones exhibited a percentage of inhibition against α-glucosidase ranging from 52 to 100%. For all these compounds, the type of inhibition and the corresponding inhibition constants (K_i's) were calculated. Accordingly, the Lineweaver–Burk plot (or double reciprocal plot) (1/v *vs.* 1/[S]) was used, where substrate concentration ranged from 1/4 K_m to 4 K_m at a fixed inhibitor concentration (2–3 different inhibitor concentrations).

As depicted in Table 3, three different types of inhibition were detected: uncompetitive (the inhibitor only binds the ES complex), mixed (the inhibitor binds both E and ES complex with two different inhibition constants, K_{ia} and K_{ib}, respectively), and non-competitive, an especial case for the mixed inhibition, where K_{ia} and K_{ib} are the same. Fig. 2a–c shows, as an example, the three modes of inhibition for compounds **12** (non-competitive), **16** (mixed) and **17** (uncompetitive).

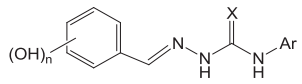
It is especially remarkable the activity found for the trihydroxylated thiosemicarbazone **12**, a potent non-competitive inhibitor, with $K_{ia} = K_{ib} = 9.6$ μM. It is noteworthy that compound **12** exhibited an 8-fold increase in activity against baker's yeast α-glucosidase compared to the pseudo-tetrasaccharide acarbose ($K_i = 77.9 \pm 11.4$ μM) [33], a pharmaceutical drug marketed by Bayer (Glucobay®) for reducing the post-prandial hyperglycemia associated to type-2 diabetes (non-insulin dependent).

Derivatives **16**–**19**, although featured with a smaller potency compared to **12**, also turned out to be moderate to good α-glucosidase inhibitors, with inhibition constants ranging from 30.3 ± 2.1 to 78.4 ± 13.9 μM. To the best of our knowledge, no previous reports on glycosidase inhibition exerted by thio- or selenosemicarbazones can be found in literature.

2.5. Antiproliferative activity

The *in vitro* antiproliferative activity of compounds **1**–**4**, **10**–**20** was evaluated using the protocol of the National Cancer Institute (NCI) of the United States [34]. Compound **9** could not be tested due to the lack of solubility under the experimental conditions. As a model, a panel of six human solid tumor cell lines was used, namely

Table 3
α-Glucosidase inhibition of compounds **10**–**20** compared to starting aldehydes **1**–**4**.



	Compound				%Inhibition at 500 μM	K_{ia}[a]	K_{ib}[b]	Type of inhibition
		$(OH)_n$	X	Ar				
Aldehydes	**1**	4-Hydroxy	–	–	N.I.[c]	–	–	–
	2	3,4-Dihydroxy	–	–	N.I.[c]	–	–	–
	3	2,4,5-Trihydroxy	–	–	N.I.[c]	–	–	–
	4	3,4,5-Trihydroxy	–	–	N.I.[c]	–	–	–
	9	–	–	–	14%	–	–	–
Thiosemicarbazones	**10**	4-Hydroxy	S	Ph	52%	–	213.0 ± 172	Uncompetitive
	11	3,4-Dihydroxy	S	Ph	68%	707.6 ± 105.5	134.4 ± 8.5	Mixed
	12	2,4,5-Trihydroxy	S	Ph	100%	9.6 ± 1.6		Non-competitive
	13	3,4,5-Trihydroxy	S	Ph	70%	297.0 ± 15.4	86.2 ± 4.7	Mixed
Selenosemicarbazones	**14**	4-Hydroxy	Se	Ph	62%	335.8 ± 138.1		Non-competitive
	15	3,4-Dihydroxy	Se	Ph	81%	201.2 ± 33.7		Non-competitive
	16	3,4,5-Trihydroxy	Se	Ph	95%	30.3 ± 2.1	78.4 ± 13.9	Mixed
	17	4-Hydroxy	Se	$C_{10}H_7$	100%	–	59.0 ± 9.2	Uncompetitive
	18	3,4-Dihydroxy	Se	$C_{10}H_7$	95%	57.8 ± 19.0	69.8 ± 18.2	Mixed
	19	2,4,5-Trihydroxy	Se	$C_{10}H_7$	95%	33.0 ± 2.4	76.8 ± 9.2	Mixed
	20	3,4,5-Trihydroxy	Se	$C_{10}H_7$	98%	153.6 ± 24.1	70.3 ± 19.9	Mixed

[a] Inhibition constant (μM) for the E–I binding.
[b] Inhibition constant (μM) for the E–S–I binding.
[c] No inhibition at 500 μM concentration.

A549 (non-small cell lung), HBL-100, (breast), HeLa (cervix), SW1573 (non-small cell lung), as drug sensitive lines, T-47D (breast) and WiDr (colon) as drug resistant lines.

Data concerning the antiproliferative activity of the tested compounds are depicted in Table 4, and are expressed as GI_{50}, that is, the concentration of the compound that inhibits 50% of the tumor cell growth. Analysis of such data allowed us to establish a series of structure-activity relationships. Thus, incorporation of a thio- or selenosemicarbazone scaffold to the phenolic framework affords a very significant increase in the antiproliferative activity, as within phenolic aldehydes, only 3,4,5-trihydroxybenzaldehyde **4** showed relevant activity against A549 cell line (GI_{50} = 7.30 ± 2.40 μM). There are also remarkable activity differences in terms of the nature of the chalcogen atom (sulfur or selenium), the number and position of the phenolic hydroxyl groups, and the aromatic *N*-substituent. In this context, selenosemicarbazones **14**–**20** were found to be stronger antiproliferative agents than the corresponding thio-isosters **10**–**13**; the difference (up to a 35-fold increase) is particularly significant for monohydroxylated derivatives.

It is also remarkable that for thiosemicarbazones **10**–**12**, and lines A549, HBL-100, SW1573 and T-47D, the order of activity was found to be **10** < **11** < **12**, what clearly indicates the importance of the phenolic skeleton, and the positive effect of adding phenolic hydroxyl groups.

Regarding selenium counterparts, most of them showed quite a potent antiproliferative activity. Thus, derivatives **15**–**20** exhibited GI_{50} values lower than 6.0 μM for all the tested cell lines. In particular, *N*-naphthyl mono- and dihydroxylated derivatives **17** and **18** had values lower than 3.3 μM (1.3–2.5 μM, 1.1–3.2 μM, respectively) for all the lines considered.

It is also noticeable the higher potency associated to *N*-naphthyl derivatives **17** and **18** when compared to *N*-phenyl counterparts **14** and **15**, bearing the same phenolic skeleton. Accordingly, the nature of the N-aryl substituent is another important factor when designing a lead anticancer phenolic Schiff base.

It is also worth mentioning that *N*-naphthyl selenosemicarbazones **17** and **18**, the derivatives featured with the strongest activity, exhibited a better antiproliferative profile against HBL-100, HeLa, SW1573, T-47D and WiDr cell lines than 5-fluorouracil and cisplatin [35], two widely-used chemotherapeutic agents (Table 4).

Compound **17** is especially relevant, with a 6.5-, 19- and 21-fold increase compared to 5-fluorouracil (HeLa, T-47D and WirD), and a 6- and 11-fold increase (T-47D, WirD), compared to cisplatin.

Therefore, these results, although preliminary, show the potential of phenolic selenosemicarbazones, for becoming lead compounds in the search for more potent anticancer drugs.

3. Conclusions

In conclusion, phenolic *N*-aryl thio- and selenosemicarbazones **10**–**20** constitute attractive templates for the development of multi-target drugs as an attempt to address multiple biological targets in the treatment of complex diseases, like cancer. Relevant *in vitro* activities (antioxidant, α-glucosidase inhibition, and antiproliferative) were found for the synthesized compounds, where some structure-activity relationships could be established regarding the number and position of phenolic hydroxyl groups, the nature of the chalcogen atom and the type of the aromatic *N*-substituent. Improved activities were found in comparison with marketed drugs, like acarbose (anti-diabetic), 5-fluorouracil, cisplatin (anticancer agents), or natural compounds like phenolic hydroxytyrosol (antioxidant found in olive oil).

4. Experimental section

4.1. Materials and methods

4.1.1. General procedures

Melting points were recorded on an Electrothermal apparatus and are uncorrected. Optical rotations were measured with a Jasco P-2000 polarimeter. ^1H (300.1 and 500.1 MHz) and ^{13}C (75.5 and 125.7 MHz) NMR spectra were recorded on Bruker Avance-300 and Avance-500 spectrometers (with a TCI cryoprobe for ^{13}C NMR spectra) at rt. The assignments of ^1H and ^{13}C signals were confirmed by homonuclear COSY and heteronuclear 2D correlated

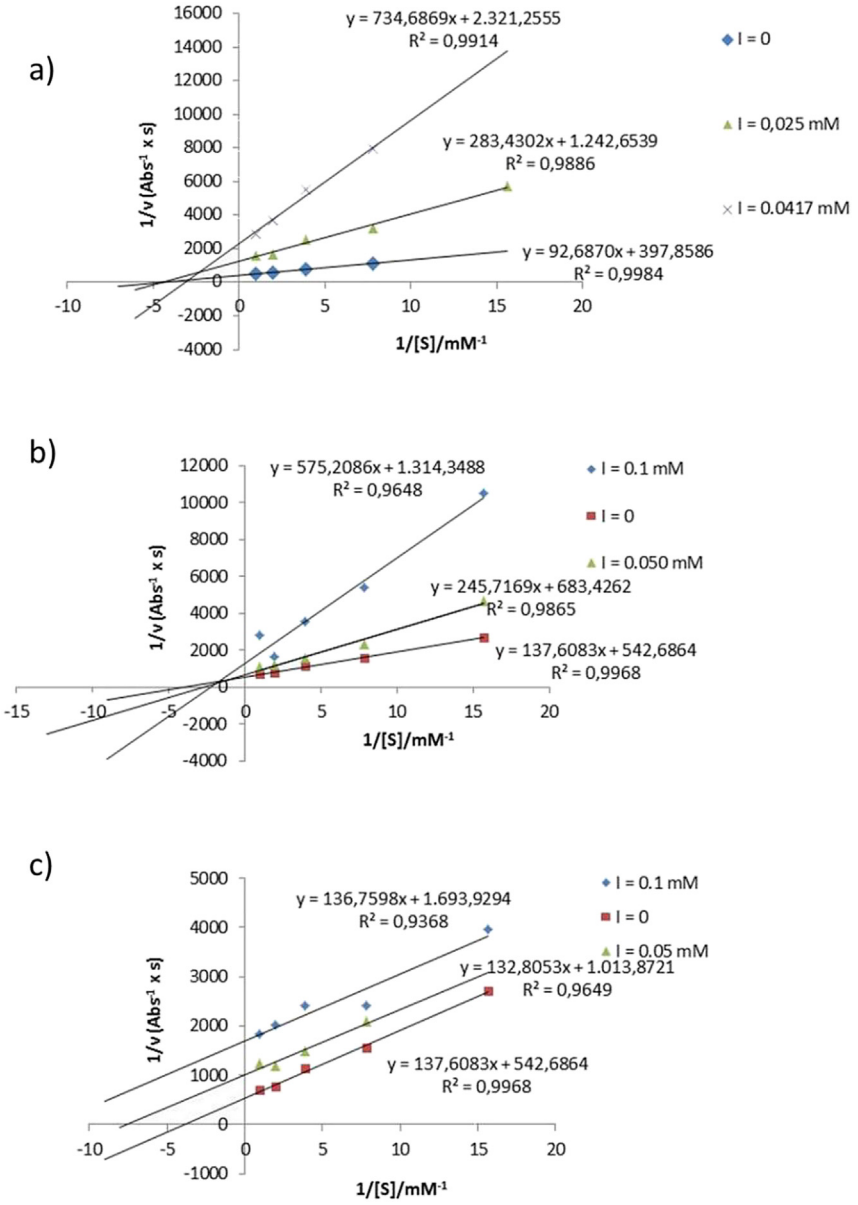


Fig. 2. Double reciprocal plots for compounds **12** (a) (non-competitive), **16** (b) (mixed) and **17** (c) (uncompetitive).

spectra, respectively. Mass spectra (CI and LSI) were recorded on Micromass AutoSpec-Q mass spectrometer with a resolution of 1000 or 10,000 (10% valley definition). For LSI spectra, ions were produced by a beam of xenon atoms and Cs^+ ions, respectively, using thioglycerol or *o*-nitrobenzyl alcohol as matrix and NaI as additive. TLCs were performed on aluminum pre-coated sheets (E. Merck Silica gel 60 F254); spots were visualized by UV light, and by charring with 10% vanillin in EtOH containing 1% of H_2SO_4, or with 3% ninhydrin in EtOH. Column chromatography was performed using E. Merck Silica Gel 60 (40–63 μm). The antioxidant assays were performed in a Hitachi U-2900 spectrophotometer with a thermostated cuvette holder, using PS cuvettes.

4.1.2. Antioxidant assays

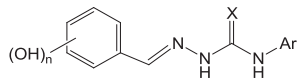
4.1.2.1. Free radical scavenging (DPPH method). The antiradical activity of the thio- and seleno derivatives was measured using commercially-available free radical DPPH, following the procedure reported by Prior et al. [19]. To a 60 μM methanolic solution of DPPH (HPLC-grade, 1.17 mL) was added the DMSO solution of the tested compounds (30 μL, 7 different concentrations), or pure DMSO (30 μL) as the control. The corresponding mixtures were kept in the dark at rt for 30 min and then, the absorbance was measured at 515 nm against a blank. Plotting the values of DPPH remaining *vs.* antioxidant concentration allows a straight line from which the EC_{50} was calculated. All the measurements were carried out in triplicate. The remaining DPPH concentration was calculated using the expression:

$$\%\text{DPPH remaining} = \frac{A_{sample}}{A_{control}} \times 100$$

A_{sample} and $A_{control}$ refer to the absorbances at 515 nm of DPPH in the sample and control solutions, respectively.

4.1.2.2. H_2O_2 scavenging activity. H_2O_2 scavenging activity was measured using the procedure reported by Bahorun et al. [23]. To a

Table 4
Antiproliferative activity (GI_{50}) against human solid tumor cells of compounds **10**–**20** compared to starting aldehydes **1**–**4**[a].



		Compound			Cell line					
		$(OH)_n$	X	Ar	A549	HBL-100	SW1573	HeLa	T-47D	WiDr
Aldehydes	**1**	4-Hydroxy	–	–	>100	>100	>100	>100	>100	>100
	2	3,4-Dihydroxy	–	–	42 ± 26	>100	54 ± 7.5	49 ± 3.1	>100	>100
	3	2,4,5-Trihydroxy	–	–	>100	>100	>100	>100	>100	>100
	4	3,4,5-Trihydroxy	–	–	7.3 ± 2.4	72 ± 7.8	38 ± 7.6	16 ± 2.1	43 ± 5.8	39 ± 5.6
Thiosemicarbazones	**10**	4-Hydroxy	S	Ph	34[b]	43 ± 1.8	35 ± 2.3	42 ± 11	88 ± 16	61 ± 12
	11	3,4-Dihydroxy	S	Ph	1.6 ± 0.4	35 ± 3.7	24 ± 9.1	7.0 ± 0.6	25 ± 10	27 ± 6.1
	12	2,4,5-Trihydroxy	S	Ph	1.2 ± 0.3	29 ± 8.1	30 ± 11	19 ± 2.7	10 ± 3.3	30 ± 5.3
	13	3,4,5-Trihydroxy	S	Ph	9.3 ± 1.9	71 ± 4.0	49 ± 4.8	32 ± 1.7	61 ± 13	>100
Selenosemicarbazones	**14**	4-Hydroxy	Se	Ph	1.4 ± 0.7	13 ± 3.1	19 ± 2.0	3.7 ± 0. 6	7.2 ± 0.8	16 ± 2.9
	15	3,4-Dihydroxy	Se	Ph	2.6 ± 0.5	3.8 ± 0.7	5.8 ± 1.3	3.3 ± 0.4	3.7 ± 0.4	3.2 ± 0.6
	16	3,4,5-Trihydroxy	Se	Ph	1.5 ± 0.3	2.0 ± 0.4	3.9 ± 0.3	2.1 ± 0.2	2.9 ± 0.6	3.7 ± 1.6
	17	4-Hydroxy	Se	$C_{10}H_7$	1.3 ± 0.01	2.1 ± 0.3	2.3 ± 0.3	2.2 ± 0.3	2.5 ± 0.4	2.3 ± 1.7
	18	3,4-Dihydroxy	Se	$C_{10}H_7$	1.1 ± 0.2	2.6 ± 0.2	3.2 ± 0.5	2.2 ± 0.3	2.5 ± 0.4	2.8 ± 0.7
	19	2,4,5-Trihydroxy	Se	$C_{10}H_7$	1.7 ± 0.2	4.0 ± 1.2	5.0 ± 1.1	3.0 ± 0.3	3.5 ± 0.6	5.2 ± 1.6
	20	3,4,5-Trihydroxy	Se	$C_{10}H_7$	1.7 ± 0.2	2.4 ± 0.6	2.9 ± 0.2	2.3 ± 0.5	2.7 ± 0.5	3.6 ± 1.0
		5-Fluorouracil [31]			–[c]	5.5 ± 2.3	4.3 ± 1.6	15 ± 4.7	47 ± 18	49 ± 6.7
		Cisplatin [31]			–[c]	1.9 ± 0.2	3.4 ± 0.7	2.0 ± 0.3	15 ± 2.3	26 ± 5.6

[a] Values are expressed in μM, and are means of two to three experiments ± standard deviation.
[b] Only one experiment.
[c] Not tested.

solution of 3×10^{-3}% H_2O_2 (100 μL), 0.1 M NaCl (100 μL) and 0.1 M phosphate buffer (pH 7.4, 700 μL) was added a 10.0 mM DMSO solution of the tested compound (100 μL), or pure DMSO (100 μL) instead of the sample, as a control. The corresponding mixture was incubated in the dark at 37 °C for 20 min. Then, a solution containing phenol red (0.2 mg/mL) and horseradish peroxidase (0.1 mg/mL) in 0.1 M phosphate buffer (pH 7.4, 1.0 mL) was added. The corresponding solution was kept at 37 °C during 15 min. Then, 1 M NaOH (100 μL) was added, and the mixture was kept at rt for 10 min; after that, the absorbance was read at 610 nm against a blank. The results are expressed as percentages of reduction of H_2O_2:

$$\%\text{Reduction } H_2O_2 = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$ and A_{sample} refer to the absorbance in the control and sample solutions, respectively.

4.1.2.3. Lipid peroxidation assay (ferric thiocyanate method, FTC). Inhibition of the lipid peroxidation was measured using the ferric thiocyanate method (FTC), following the procedure reported by Olszewska et al. [25].

To a 10.0 mM DMSO solution (37 μL) of the tested compounds and 1.3% (w/v) methanolic linoleic acid (175 μL) in H_2O (88 μL) and 0.2 M phosphate buffer (pH 7.0, 175 μL) in a screw-cap vial was added AAPH in buffer (55.3 mM, 25 μL). The control solution was prepared by adding pure DMSO (37 μL), instead of the sample. The vial was incubated at 50 ± 0.1 °C for 24 h in the dark. After that, an aliquot (30 μL) of the reaction mixture was dissolved in a 2.7:1H_2O–DMSO mixture (1.1 mL), and a 10% aqueous solution of NH_4SCN (30 μL) and 20 mM $FeCl_2$ in 3.5% HCl (30 μL) were added; after 3 min of incubation at rt, the absorbance was measured at 546 nm against the corresponding blank. The results are expressed as the percentage of lipid peroxidation inhibition:

$$\%\text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$ and A_{sample} refer to the absorbance of the control and sample solutions, respectively.

4.1.3. Pro-oxidant activity

The analysis of the pro-oxidant activity [28] was accomplished by using a modification of the original procedure reported [36] by Halliwell et al. for determining the concentration of hydroxyl radicals. A mixture of 0.1 M phosphate buffer (pH 7.4, 0.14 mL), 33.6 mM 2-deoxyribose (0.1 mL), 33.6 mM H_2O_2 (0.1 mL), 0.27 mM $FeCl_3$ (0.1 mL), 1.2 mM EDTA (in phosphate buffer, 0.1 mL), sample (0.1 mL) and water (0.56 mL) was heated at 37 °C for 1 h. The $FeCl_3$ and EDTA solutions were premixed prior to the addition to the mixture. After that, 1% (w/v) TBA in 0.05 M NaOH (1.0 mL) and 2.8% (w/v) trichloroacetic acid (1.0 mL) were added and the corresponding mixture was heated at 100 °C in a water bath for 15 min. Finally, after cooling down to rt, the absorbance was measured at 532 nm against the appropriate blank solution. For the control experiment, the sample solution was replaced with pure DMSO (0.1 mL).

4.1.4. α-Glucosidase inhibition

The enzymatic inhibition assay was accomplished following the method reported by Bols and co-workers [37]. The percentage of inhibition was measured by preparing two 1.2-mL samples in PS cuvettes containing 0.1 M phosphate buffer (pH 6.8) and *p*-nitrophenyl-α-D-glucopyranoside at a concentration equal to the value of K_m. DMSO or inhibitor solution (500 μM final concentration in DMSO) plus water were added up to a constant volume of 1.14 mL (control or inhibitor solution, respectively). Reaction was started by adding 60 μL of properly diluted enzyme solution at 25 °C and the formation of the *p*-nitrophenolate was monitored for 125 s by measuring the increase of absorbance at 400 nm. Initial rates were calculated from the slopes of both plots (Abs *vs.* t) and were used for

calculating the percentage of inhibition:

$$\%\text{Inhibition} = \frac{v_o - v}{v_o} \times 100$$

v_o and v refer to reaction rates for the enzyme and enzyme plus inhibitor solutions.

DMSO concentration was maintained at 5% (v/v) of the total assay mixture; under these conditions, no appreciable effect of DMSO was observed on α-glucosidase activity. When a percentage of inhibition over 50% was observed, the mode of inhibition and the inhibition constants (K_i) were determined. In this case, a similar assay was conducted, but using 5 different substrate concentrations, ranging from 0.25 to 4.0 K_m at a fixed inhibitor concentration; 2–3 different inhibitor concentrations were used for estimating the mode of inhibition. For this purpose, the Lineweaver–Burk, or double reciprocal plot, was used (1/v *vs.* 1/[S]).

4.1.5. Antiproliferative activity

The human solid tumor cell lines A549, HBL-100, HeLa, SW1573, T-47D and WiDr were used herein. Cells were maintained in 25 cm^3 culture flasks in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum and 2 mM L-glutamine in a 37 °C, 5% CO_2, 95% humidified air incubator. Exponentially growing cells were trypsinized and re-suspended in antibiotic containing medium (100 units penicillin G and 0.1 mg of streptomycin per mL). Single cell suspensions displaying >97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto 96-well microtiter plates. Cells were inoculated in a volume of 100 mL per well at densities of 10,000 (A549, HBL-100, HeLa and SW1573), 15,000 (T-47D), and 20,000 (WiDr) cells per well, based on their doubling times.

Compounds to be tested were dissolved in DMSO at an initial concentration of 40 mM; derivative **9** was not soluble under the experimental conditions, so it was discarded. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, negative control). Each agent was tested in triplicate at different dilutions in the range of 1–100 μM. The drug treatment was started on day 1 after plating. Drug incubation times were 48 h, after which time cells were precipitated with 25 mL ice-cold TCA (50% w/v) and fixed for 60 min at 4 °C. Then the sulforhodamine B (SRB) assay was performed [38]. The optical density (OD) of each well was measured at 492 nm, using BioTek's PowerWave XS Absorbance Microplate Reader. Values were corrected for background OD from wells only containing medium.

4.1.6. Statistical analysis

For antioxidant and glycosidase inhibition assays, all tests were run in triplicate. Values are expressed as the confidence interval, which was calculated for P = 0.95 using the Student's *t*-distribution. For antiproliferative assays, the median and standard deviation (SD) for 2–3 measurements were calculated.

4.2. Chemistry

4.2.1. 4-α-Naphthylselenosemicarbazide (**9**)

A solution of α-naphthyl isoselenocyanate **7** (500 mg, 2.15 mmol) and 85% hydrazine monohydrate (0.10 mL, 2.15 mmol) in CH_2Cl_2 (25 mL) was stirred in the dark at rt and under inert atmosphere for 1 h. Removal of the solvent and crystallization (Et_2O) afforded **9** as a white solid. Yield: 522 mg, 92%; mp > 210 °C (Et_2O); R_f: 0.46 (EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.66 (s, 1H, NH), 7.97–7.94 (m, 1H, Ar–H), 7.88–7.83 (m, 2H, Ar–H), 7.59–7.48 (m, 5H, 4Ar–H, Ar–N*H*), 6.55 (brs, 2H, NH_2); ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 178.4 (CS), 136.2, 133.6, 130.1, 128.0, 126.3, 125.9 (x2), 125.8, 125.2, 123.0 (Ar–C); CI-MS *m*/*z* 265 ($[M]^+$, 9%); HRCI-MS calcd. for $C_{11}H_{11}N_3Se$ ($[M]^+$):265.0118, found: 265.0111.

4.2.2. General procedure for the preparation of thio- and selenosemicarbazones ***10–20***

To a solution of the aryl thio- or selenosemicarbazide **5**, **8** or **9** (0.4 mmol) in EtOH (4 mL) were added the corresponding aldehyde (0.4 mmol), AcOH (50 μL, 3.0 mmol) and water (0.5 mL), and the corresponding mixture was refluxed, or heated at 45 °C (compounds **18** and **20**) under inert atmosphere for 24 h. After that, the solvent was removed *in vacuo* and the residue was purified as indicated in each case.

*4.2.2.1. 1-(3′,4′-Dihydroxybenzylidene)-4-phenyl-3-thiosemicarbazone (**11**).* 3,4-Dihydroxybenzaldehyde (55.2 mg, 0.4 mmol) and 4-phenylthiosemicarbazide (67 mg, 0.4 mmol) were used. Column chromatography (cyclohexane→1:1 cyclohexane–EtOAc) afforded **11** as a yellow solid. Yield: 63 mg, 55%; mp 190 °C (dec.) (Et_2O); R_f: 0.30 (1:2 EtOAc–cyclohexane). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.60 (s, 1H, NH), 9.93 (s, 1H, Ar–NH), 9.53, 9.00 (2s, 1H each, 2OH), 8.00 (s, 1H, N=CH), 7.60 (m, 2H, Ar–H*o*, Ph), 7.37 (m, 2H, Ar–H*m*, Ph), 7.36 (d,1H, $J_{3',6'} = 2.0$ Hz, H-2′), 7.23 (m, 1H, Ar–H*p*, Ph), 7.14 (dd, 1H, $J_{5',6'} = 8.3$ Hz, H-6′), 6.79 (d, 1H, H-5′); ^{13}C-RMN (75.5 MHz, DMSO-d_6) δ 173.1 (CS), 148.2, 145.6 (C-3′, C-4′), 145.2 (Ar–C), 139.8 (C=N), 128.0, 126.4, 125.6, 125.2, 120.9 (Ar–C), 115.5, 114.3 (C-2′, C-5′); CI-MS *m*/*z* 287 ($[M]^+$, 24%); HRCI-MS calcd. for $C_{14}H_{13}N_3O_2S$ ($[M]^+$): 287.0728, found: 287.0724.

*4.2.2.2. 4-Phenyl-1-(3′,4′,5′-trihydroxybenzylidene)-3-thiosemicarbazone (**13**).* 3,4,5-Trihidroxybenzaldehyde (61.6 mg, 0.4 mmol) and 4-phenylthiosemicarbazide (67 mg, 0.4 mmol) were used. Column chromatography (cyclohexane→2:1 cyclohexane–EtOAc) afforded **13** as a yellow solid. Yield: 58 mg, 48%; mp 158 °C (dec.) (Et_2O); R_f: 0.40 (1:1 EtOAc–cyclohexane); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.56 (s, 1H, NH), 9.88 (s, 1H, Ar–NH), 9.02 (s, 2H, 2OH), 8.70 (s, 1H, OH), 7.92 (s, 1H, N=CH), 7.59 (m, 2H, Ar–H*o*), 7.35 (m, 2H, Ar–H*m*), 7.18 (m, 1H, Ar–H*p*), 6.79 (s, 2H, H-2′, H-6′); ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 175.2 (CS), 146.2 (C-3′, C-5′), 144.2 (C-4′), 139.1 (N=C), 136.0 (Ar–C *ipso*), 128.1 (x2) (Ar–C*m*), 125.2 (x2) (Ar–C*o*), 125.0 (Ar–C), 124.2 (Ar–C), 107.0 (x2) (C-2′, C-6′); CI-MS *m*/*z* 303 ($[M]^+$, 13%); HRCI-MS calcd for $C_{14}H_{13}N_3O_3S$ ($[M]^+$): 303.0678, found: 303.0664.

*4.2.2.3. 1-(4′-Hydroxybenzylidene)-4-phenyl-3-selenosemicarbazone (**14**).* 4-Hydroxybenzaldehyde (48.8 mg, 0.4 mmol) and 4-phenylselenosemicarbazide (**8**) (66.9 mg, 0.4 mmol) were used. Column chromatography (cyclohexane→2:1 cyclohexane–AcOEt) afforded **14** as a brown solid. Yield: 53 mg, 42%; mp 136 °C (dec.) (Et_2O); *R*f: 0.59 (3:1 Et_2O-cyclohexane); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.95 (s, 1H, NH), 10.32 (s, 1H, OH), 9.96 (s, 1H, Ar–NH), 8.18 (s, 1H, N=CH), 7.75 (m, 2H, H-2′, H-6′), 7.53 (m, 2H, Ar–H*o*), 7.37 (m, 2H, Ar–H*m*), 7.23 (m, 1H, Ar–H*p*), 6.81 (m, 2H, H-3′, H-5′); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 173.3 (CS), 159.7 (C-4′), 144.7 (Ar–C *ipso*), 139.9 (N=C), 129.7 (x2) (C-2′, C-6′), 128.0 (x2) (Ar–C*m*), 126.6 (x2) (Ar–C), 125.7 (C-1′), 124.8 (Ar–C*p*), 115.6 (x2) (Ar–C); CI-MS *m*/*z* 320 ($[M+H]^+$, 10%); HRCI-MS calcd for $C_{14}H_{14}N_3O^{80}Se$ ($[M+H]^+$): 320.0302; found: 320.0302.

*4.2.2.4. 1-(3′,4′-Dihydroxybenzylidene)-4-phenyl-3-selenosemicarbazone (**15**).* 3,4-Dihydroxybenzaldehyde (55.2 mg, 0.4 mmol) and 4-phenylselenosemicarbazide (**8**) (66.9 mg, 0.4 mmol) were used. Column chromatography (cyclohexane→1:2 cyclohexane–EtOAc) afforded **15** as a yellow solid. Yield: 96 mg, 72%; mp 119 °C (dec.) (Et_2O); R_f: 0.59 (3:1 Et_2O–cyclohexane); ^{1}H

NMR (300 MHz, DMSO-d_6) δ 11.90 (s, 1H, NH), 10.27 (s, 1H, Ar–NH), 9.55, 9.02 (2brs, 1H each, 2OH), 8.12 (s, 1H, N=CH), 7.54 (m, 2H, Ar–H*o*, Ph), 7.37 (m, Ar–H*m*, Ph), 7.35 (d, 1H, $J_{2',6'} = 2.2$ Hz, H-2′), 7.22 (m, 1H, Ar-H*p*, Ph), 7.13 (dd, 1H, $J_{5',6'} = 8.2$ Hz, H-6′), 6.78 (d, 1H, H-5′); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 173.1 (CSe), 148.2, 145.6 (C-3′, C-4′), 145.2 (Ar–C), 139.9 (C=N), 128.0, 126.4, 125.6, 125.2, 120.9 (Ar–C), 115.5, 114.3 (C-2′, C-5′); CI-MS *m*/*z* 335 ([M]$^+$, 2%); HRCI-MS calcd for $C_{14}H_{13}N_3O_2^{80}Se$ ([M]$^+$): 335.0173, found: 335.0180.

*4.2.2.5. 4-Phenyl-1-(3′,4′,5′-Trihydroxybenzylidene)-3-selenosemicarbazone (**16**).* 3,4,5-Trihydroxybenzaldehyde (68.9 mg, 0.4 mmol) and 4-phenylselenosemicarbazide (**8**) (66.9 mg, 0.4 mmol) were used. Column chromatography (cyclohexane → 1:2 cyclohexane–EtOAc) afforded **16** as an orange solid. Yield: 39 mg, 28%; mp 148 °C (dec.) (Et_2O); R_f: 0.27 (1:1 EtOAc-cyclohexane); ^{1}H-RMN (300 MHz, DMSO-d_6) δ 11.85 (s, 1H, NH), 10.22 (s, 1H, Ar–NH), 9.07–8.70 (m, 3H, 3OH), 8.03 (s, 1H, N=CH), 7.54 (m, 2H, Ar–H*o*, Ph), 7.36 (m, 2H, Ar–H*m*, Ph), 7.22 (m, 1H, Ar–H*p*, Ph), 6.81 (s, 2H, H-2′, H-6′); ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 173.0 (CSe), 146.1, 145.9, 145.6 (C-3′, C, 4′, C-5′), 139.9 (C=N), 136.2 (Ar–C *ipso*), 128.0 (x2) (Ar–C*m*), 126.2 (x2) (Ar–C*o*), 125.6 (Ar–C), 124.1 (Ar–C), 107.2 (x2) (C-2′, C-6′); CI-MS *m*/*z* 353 ([M + H]$^+$, 8%); HRCI-MS calcd for $C_{14}H_{14}N_3O_3^{80}Se$ ([M]$^+$): 352.0200, found: 352.0193.

*4.2.2.6. 1-(4′-Hydroxybenzylidene)-4-(α-naphthyl)-3-selenosemicarbazone (**17**).* 4-Hydroxybenzaldehyde (48.8 mg, 0.4 mmol) and 4-α-naphthylselenocarbazide (**9**) (105.7 mg, 0.4 mmol) were used. Column chromatography (cyclohexane → 1:2 cyclohexane–EtOAc) afforded **17** as a yellow solid. Yield: 64 mg, 43%; mp 153 °C (dec.) (Et_2O); R_f: 0.44 (1:2 EtOAc–cyclohexane); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.08 (s, 1H, NH), 10.61 (s, 1H, OH), 9.94 (s, 1H, Ar–NH), 8.23 (s, 1H, N=CH), 7.98 (dd, 1H, $J_{6'',8''} = 3.1$ Hz, $J_{7'',8''} = 6.2$ Hz, H-8″), 7.91 (brd, 1H, $J_{5'',6''} = 8.0$ Hz, H-5″), 7.84 (dd, 1H, $J_{H,H} = 3.4$ Hz, $J_{H,H} = 6.2$ Hz, Ar–H), 7.78 (m, 2H, H-2′, H-6′), 7.58–7.49 (m, 4H, Ar–H, naphthyl), 6.79 (m, 2H, H-3′, H-5′); ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 175.3 (CSe), 169.6 (C-4′), 144.5 (C-1″), 136.7 (N=C), 133,7, 130.6 (Ar–C), 129.7 (x2) (C-2′, C-6′), 127.9, 127.0, 126.8, 126.0 (x2), 125.4, 125.0, 123.5 (Ar–C), 115.6 (x2) (C-3′, C-5′); LSI-MS *m*/*z* 370 ([M + H]$^+$, 5%); HRLSI-MS calcd for $C_{18}H_{16}N_3O^{80}Se$ ([M + H]$^+$): 370.0459, found: 370.0460.

*4.2.2.7. 1-(3′,4′-Dihydroxybenzylidene)-4-(α-naphthyl)-3-selenosemicarbazone (**18**).* 3,4-Dihydroxybenzaldehyde (55.2 mg, 0.4 mmol) and 4-α-naphthylselenocarbazide (**9**) (105.7 mg, 0.4 mmol) were used. Recrystallization from Et_2O afforded **18** as a yellow solid. Yield: 139 mg, 90%; mp 173 °C (dec.) (Et_2O); R_f: 0.72 (5:1 Et_2O-cyclohexane); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.02 (s, 1H, NH), 10.58 (s, 1H, Ar–NH), 9.60, 8.95 (2s, 1H each, 2OH), 8.16 (s, 1H, N=CH), 7.99–7.96 (m, 1H, H-8″), 7.90 (brd, 1H, $J_{5'',6''} = 7.9$ Hz, H-5″), 7.86–7.83 (m, 1H, Ar–H), 7.56–7.51 (m, 4H, Ar–H), 7.39 (d, 1H, $J_{2',6'} = 1.9$ Hz, H-2′), 7.16 (dd, 1H, $J_{5',6'} = 8.2$ Hz, H-6′), 6.77 (d, 1H, H-5′); ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 175.2 (CSe), 148.1, 145.6 (C-3′, C-4′), 145.0 (N=C), 136.7, 133.7, 130.5 (C-1′, C-1″, C-4″a), 127.9 (C-8″), 127.0 (C-5″), 126.7, 126.0, 125.4, 125.3, 123.5 (Ar–C), 120.9 (C-6′), 115.4 (C-5′), 114.4 (C-2′); LSI-MS *m*/*z* 385 ([M]$^+$, 3%); HRLSI-MS calcd for $C_{18}H_{15}N_3O_2^{80}Se$ ([M]$^+$): 385.0329, found: 385.0331.

*4.2.2.8. 4-(α-Naphthyl)-1-(2′,4′,5′-trihydroxybenzylidene)-3-selenosemicarbazone (**19**).* 2,4,5-Trihydroxybenzaldehyde (61.6 mg, 0.4 mmol) and 4-α-naphthylselenocarbazide (**9**) (105.7 mg, 0.4 mmol) were used. Column chromatography (cyclohexane → 1:1 cyclohexane–EtOAc) afforded **19** as a yellow solid. Yield: 89 mg, 56%; mp 165 °C (dec.) (Et_2O); R_f: 0.58 (2:1 Et_2O-cyclohexane); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.96 (s, 1H, NH), 10.51 (s, 1H, Ar–NH), 9.62, 9.23, 8.49 (3s, 1H each, 3OH), 8.18 (s, 1H, N=CH), 7.99–7.96 (m, 1H, H-8 naphth), 7.89 (brd, 1H, $J_{5,6} = 7.7$ Hz, H-5 naphth), 7.86–7.83 (m, 1H, Ar–H), 7.57–7.51 (m, 4H, Ar–H), 7.41 (s, 1H, H-6′), 6.35 (s, 1H, H-3′); ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 174.5 (CSe), 151.3, 149.6 (C-2′, C-4′), 142.7 (N=C), 138.7, 136.8, 133.7, 130.6, 127.9, 126.9, 126.7, 126.0, 125.4, 123.4, 113.3, 110.7, 103.2 (Ar–C).

*4.2.2.9. 4-(α-Naphthyl)-1-(3′,4′,5′-trihydroxybenzylidene)-3-selenosemicarbazone (**20**).* 3,4,5-Trihydroxybenzaldehyde (61.6 mg, 0.4 mmol) and 4-α-naphthylselenocarbazide (**9**) (105.7 mg, 0.4 mmol) were used. Recrystallization from Et_2O afforded **20** as an orange solid. Yield: 148 mg, 93%; mp 153 °C (dec.) (Et_2O); R_f: 0.56 (5:1 Et_2O-cyclohexane); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.98 (s, 1H, NH), 10.55 (s, 1H, Ar–NH), 9.00 (s, 2H, 2OH), 8.76 (s, 1H, OH), 8.08 (s, 1H, N=CH), 7.99–7.96 (m, 1H, H-8 naphth), 7.90 (brd, 1H, $J_{5,6} = 7.7$ Hz, H-5 Naphth), 7.86–7.83 (m, 1H, Ar–H), 7.57–7.49 (m, 4H, Ar–H), 6.86 (s, 2H, H-2′, H-6′); ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 175.1 (CSe), 146.1 (x2) (C-3′, C-5′), 145.5 (C-4′), 136.7 (N=C), 136.2, 133.7, 130.5, 128.0, 127.0, 126.7, 126.0 (x2), 125.4, 124.3, 123.4 (Ar–C), 107.3 (x2) (C-2′, C-6′); LSI-MS *m*/*z* 402 ([M + H]$^+$, 5%); HRLSI-MS calcd for $C_{18}H_{16}N_3O_3^{80}Se$ ([M + H]$^+$): 402.0357, found: 402.0363.

Acknowledgments

We thank the Dirección General de Investigación of Spain (CTQ2008-02813, CTQ2011-28417-C02-01, CEI10/00018), the Junta de Andalucía (FQM134), the Instituto de Salud Carlos III (PI11/00840), the European Regional Development Fund (FEDER), and the EU Research Potential (FP7-REGPOT-2012-CT2012-31637-IMBRAIN), for financial support. G.B.P. also thanks the Obra Social La Caixa-Fundación Caja Canarias for a predoctoral grant.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.02.037.

References

[1] S. Matsunaga, M. Shibasaki, Recent advances in cooperative bimetallic asymmetric catalysis: dinuclear schiff base complexes, Chem. Commun. 50 (2014) 1044–1057.
[2] L.E. Santos-Figueroa, M.E. Moragues, M.M.M. Raposo, R.M.F. Batista, S.P.G. Costa, R.C.M. Ferreira, F. Sancenón, R. Martínez-Máñez, J.V. Ros-Lis, J. Soto, Synthesis and evaluation of thiosemicarbazones functionalized with furyl moieties as new chemosensors for anion recognition, Org. Biomol. Chem. 10 (2012) 7418–7428.
[3] C. García-Iriepa, M. Marazzi, L.M. Frutos, D. Sampedro, *E*/*Z* photochemical switches: syntheses, properties and applications, RSC Adv. 3 (2013) 6241–6266.
[4] A.K. Mahapatra, J. Roy, P. Sahoo, S.K. Mukhopadhyay, A. Chattopadhyay, Carbazole–thiosemicarbazone–Hg(II) ensemble-based colorimetric and fluorescence turn-on toward iodide in aqueous media and its application in live cell imaging, Org. Biomol. Chem. 10 (2012) 2231–2236.
[5] Z.H. Chohan, S.H. Sumrra, M.H. Youssoufi, T.B. Hadda, Metal based biologically active compounds: design, synthesis, and antibacterial/antifungal/cytotoxic properties of triazole-derived Schiff bases and their oxovanadium(IV) complexes, Eur. J. Med. Chem. 45 (2010) 2739–2747.
[6] D.J.R. Lane, T.M. Mills, N.H. Shafie, A.M. Merlot, R.S. Moussa, D.S. Kalinowski, Z. Kovacevic, D.R. Richardson, Expanding horizons in iron chelation and the treatment of cancer: role of iron in the regulation of ER stress and the epithelial–mesenchymal transition, Biochim. Biophys. Acta 1845 (2014) 166–181.
[7] M.A.G.B. Gomes, L.P. Carvalho, B.S. Rocha, R.R. Oliveira, E.J.T. de Melo, E.J. Maria, Evaluating anti-*Toxoplasma gondii* activity of new serie of phenylsemicarbazone and phenylthiosemicarbazones in vitro, Med. Chem. Res. 22 (2013) 3574–3580.
[8] L. Blau, R.F. Menegon, G.H.G. Trossini, J.V.D. Molino, D.G. Vital, R.M.B. Cicarelli, G.D. Passerini, P.L. Bosquesi, C.M. Chin, Design, synthesis and biological evaluation of new aryl thiosemicarbazone as antichagasic candidates, Eur. J. Med. Chem. 67 (2013) 142–151.
[9] M.A.M.Sh El-Sharief, S.Y. Abbas, K.A.M. El-Bayouki, E.W. El-Gammal, Synthesis of thiosemicarbazones derived from *N*-(4-hippuric acid)thiosemicarbazide

and different carbonyl compounds as antimicrobial agents, Eur. J. Med. Chem. 67 (2013) 263–268.
[10] M. Leigh, D.J. Raines, C.E. Castillo, A.K. Duhme-Klair, Inhibition of xanthine oxidase by thiosemicarbazones, hydrazones and dithiocarbazates derived from hydroxy-substituted benzaldehydes, ChemMedChem 6 (2011) 1107–1118.
[11] Y. Bansal, O. Silakari, Multifunctional compounds: smart molecules for multifactorial diseases, Eur. J. Med. Chem. 76 (2014) 31–42.
[12] a) G.K. Azad, R.S. Tomar, Ebselen, a promising antioxidant drug: mechanisms of action and targets of biological pathways, Mol. Biol. Rep. 41 (2014) 4865–4879;
b) S.P.J.N. Senanayake, Green tea extract: chemistry, antioxidant properties and food applications – a review, J. Funct. Foods 5 (2013) 1529–1541.
[13] a) D.-Y. Choi, Y.-J. Lee, J.T. Hong, H.-J. Lee, Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease, Brain Res. Bull. 87 (2012) 144–153;
b) R. Naithani, Organoselenium compounds in cancer chemoprevention, Mini. Rev. Med. Chem. 8 (2008) 657–668.
[14] Y. Xie, J. Liu, J. Li, Selective synthesis of novel 2-imino-1,3-selenazolidin-4-ones and 2-amino-1,3,4-selenadiazin-5-ones from isoselenocyanates, Tetrahedron Lett. 52 (2011) 932–935.
[15] Ó. López, S. Maza, V. Ulgar, I. Maya, J.G. Fernández-Bolaños, Synthesis of sugar-derived isoselenocyanates, selenoureas, and selenazoles, Tetrahedron 65 (2009) 2556–2566.
[16] R. Kohen, A. Nyska, Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification, Toxicol. Pathol. 30 (2002) 620–650.
[17] a) J. Li, W. O, W. Li, Z.-G. Jiang, H.A. Ghanbari, Oxidative stress and neurodegenerative disorders, Int. J. Mol. Sci. 14 (2013) 24438–24475;
b) V.M. Costa, F. Carvalho, J.A. Duarte, M.L. Bastos, F. Remião, The heart as a target for xenobiotic toxicity: the cardiac susceptibility to oxidative stress,, Chem. Res. Toxicol. 26 (2013) 1285–1311.
[18] A. Mencalha, V.J. Victorino, R. Cecchini, C. Panis, Mapping oxidative changes in breast cancer: understanding the basic to reach the clinics, Anticancer Res. 34 (2014) 1127–1140.
[19] R.L. Prior, X. Wu, K. Schaich, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, J. Agric. Food Chem. 53 (2005) 4290–4302.
[20] J.G. Fernández-Bolaños, Ó. López, J. Fernández-Bolaños, G. Rodríguez-Gutiérrez, Hydroxytyrosol and derivatives: isolation, synthesis, and biological properties, Cur. Org. Chem. 12 (2008) 442–463.
[21] C. Virruso, G. Accardi, G. Colonna-Romano, G. Candore, S. Vasto, C. Caruso, Nutraceutical properties of extra-virgin olive oil: a natural remedy for age-related disease? Rejuvenation Res. 17 (2014) 217–220.
[22] A. Boveris, E. Cadenas, Mitochondrial production of hydrogen peroxide-regulation by nitric oxide and the role of ubisemiquinone, IUBMB Life 50 (2000) 245–250.
[23] T. Bahorun, B. Gressier, F. Trotin, C. Brunet, T. Dine, M. Luyckx, J. Vasseur, M. Cazin, J.C. Cazin, M. Pinkas, Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations, Arzheim. Forsch. 46 (1996) 1086–1089.
[24] J. Wong-ekkabut, Z. Xu, W. Triampo, I.-M. Tang, D.P. Tieleman, L. Monticelli, Effect of lipid peroxidation on the properties of lipid bilayers: a molecular dynamics study, Biophys. J. 93 (2007) 4225–4236.
[25] M.A. Olszewska, A. Presler, P. Michel, Profiling of phenolic compounds and antioxidant activity of dry extracts from the selected *Sorbus* species, Molecules 17 (2012) 3093–3113.
[26] a) T. Bakir, I. Sönmezoğlu, F. Imer, R. Apak, Antioxidant/prooxidant effects of α-tocopherol, quercetin and isorhamnetin on linoleic acid peroxidation induced by Cu(II) and H_2O_2, Int. J. Food Sci. Nutr. 65 (2014) 226–234;
b) A. Banerjeea, A. Kunwarb, B. Mishrab, K.I. Priyadarsini, Concentration dependent antioxidant/pro-oxidant activity of curcumin studies from AAPH induced hemolysis of RBCs, Chem. Biol. Interact. 174 (2008) 134–139;
c) G.-C. Yen, P.-D. Duh, H.-L. Tsai, Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid, Food Chem. 79 (2002) 307–313.
[27] M.C. Putchala, P. Ramani, H.J. Sherlin, P. Premkumar, A. Natesan, Ascorbic acid and its pro-oxidant activity as a therapy for tumours of oral cavity – a systematic review, Arch. Oral Biol. 58 (2013) 563–574.
[28] E. Joubert, P. Winterton, T.J. Britz, W.C.A. Gelderblom, Antioxidant and pro-oxidant activities of aqueous extracts and crude polyphenolic fractions of rooibos (*Aspalathus linearis*), J. Agric. Food Chem. 53 (2005) 10260–10267.
[29] W. Rosenblum, F. El-Sabban, Dimethyl sulfoxide (DMSO) and glycerol, hydroxyl radical scavengers, impair platelet aggregation within and eliminate the accompanying vasodilation of, injured mouse pial arterioles, Stroke 13 (1982) 35–39.
[30] a) Ó. López, P. Merino-Montiel, S. Martos, A. González-Benjumea, in: A.P. Rauter, T. Lindhorst (Eds.), Glycosidase Inhibitors: Versatile Tools in Glycobiology, in Carbohydrate Chemistry-chemical and Biological Approaches: Specialist Periodical Reports, vol. 38, Royal Society of Chemistry, 2012, pp. 215–262;
b) M. Bols, Ó. López, F. Ortega-Caballero, in: J.P. Kamerling (Ed.), Glycosidase Inhibitors: Structure, Activity, Synthesis, and Medical Relevance, in Comprehensive Glycoscience-from Chemistry to Systems Biology, vol. 1, Elsevier, 2007, pp. 815–884.
[31] S.-I. Hakomori, Aberrant glycosylation in cancer cell membranes as focused on glycolipids: overview and perspectives, Cancer Res. 45 (1985) 2405–2414.
[32] J. Xiao, G. Kai, K. Yamamoto, X. Chen, Advance in dietary polyphenols as α-glucosidases inhibitors: a review on structure-activity relationship aspect, Crit. Rev. Food Sci. Nutr. 53 (2013) 818–836.
[33] M.-J. Kim, S.-B. Lee, H.-S. Lee, S.-Y. Lee, J.-S. Baek, D. Kim, T.-W. Moon, J.F. Robyt, K.-H. Park, Comparative study of the inhibition of α-glucosidase, α-amylase, and cyclomaltodextrin glucanosyltransferase by acarbose, isoacarbose, and acarviosine–glucose, Arch. Biochem. Biophys. 371 (1999) 277–283.
[34] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, J. Natl. Cancer Inst. 82 (1990) 1107–1112.
[35] M.E. García, G.E. Barboza, J.C. Oberti, C. Ríos-Luci, J.M. Padrón, V.E. Nicotra, A. Estévez-Braun, A.G. Ravelo, Antiproliferative activity of withanolide derivatives from Jaborosa cabrerae and Jaborosa reflexa. Chemotaxonomic considerations, Phytochemistry 76 (2012) 150–157.
[36] B. Halliwell, J.M.C. Gutteridge, O.I. Aruoma, The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals, Anal. Biochem. 165 (1987) 215–219.
[37] M. Bols, R.G. Hazell, I.B. Thomsen, 1-Azafagomine-a hydroxyhexahydropyridazine that potently inhibits enzymatic glycoside cleavage, Chem. Eur. J. 3 (1997) 940–947.
[38] P.O. Miranda, J.M. Padrón, J.I. Padrón, V.S. Martín, Prins-type synthesis and SAR study of cytotoxic alkyl chloro dihydropyrans, ChemMedChem 1 (2006) 323–329.