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Design, synthesis and evaluation of seleno-dihydropyrimidinones as potential multi-targeted therapeutics for Alzheimer's disease

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In this paper we report the design, synthesis and evaluation of a series of seleno-dihydropyrimidinones as potential multi-targeted therapeutics for Alzheimer's disease. The compounds show excellent results as acetylcholinesterase inhibitors, being as active as the standard drug. All compounds also show very good antioxidant activity through different mechanisms of action.

Introduction

Alzheimer's disease (AD), the most prevalent of the neurodegenerative diseases, affects approximately 15 million people worldwide and nearly 50% of adults over the age of 85.[1]

Because of the complex pathophysiology of AD, which involves many pathways, the development of a satisfactory therapy is problematic. The main therapy targets are reduced levels of the neurotransmitter acethylcholine (ACh), diffuse loss of neurons, neurofibrillary tangles and deposits of β-amyloid (Aβ) plaques.[2] Based on the cholinergic hypothesis, the mainstays of the current pharmacotherapy for AD are drugs aimed at increasing the levels of ACh through the inhibition of cholinesterases (ChEs).[3] ChE inhibitors have been approved as an efficacious treatment to reduce the symptoms of the early stage of AD. Several anticholinesterase agents, such as tacrine,[4] ensaculin,[5] donepezil[6] and galanthamine,[7] have been shown to induce modest improvements in relation to memory and cognitive function. Recent evidence suggests that acethylcholinesterase (AChE) also plays a non-cholinergic role in the development of AD,[8] as it works as a chaperone, accelerating the Aβ peptide deposition and the aggregation of Aβ into insoluble fibrils.[9]

Another of the current therapeutic strategies to treat AD is to reduce the oxidative stress involved in cellular death. Because of its high oxygen consumption rate, high lipid content, and relatively limited antioxidant capacity compared to other organs, the brain is particularly susceptible to oxidative damage. Senile plaques release free radicals that are extremely toxic.[10] The accumulation of reactive oxygen species (ROS) results in damage to major cell components, such as DNA, membranes and cytoplasmic proteins.[11] Oxidative stress is therefore included in all the pathophysiological hypotheses for AD, and studies have shown the efficacy of several antioxidant compounds.[12]

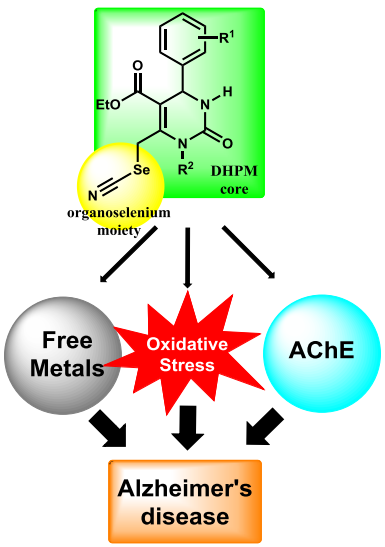


Figure 1

In addition to these two hypotheses, a third, known as the metal hypothesis, considers that metals (Fe and Cu) also play a role in the pathogenesis of AD.[13] During the disease progression, metals progressively accumulate in the cerebrum.[14] The abnormal accumulation of metals is closely associated with the formation of Aβ plaques and neurofibrillary tangles.[15] In addition, abnormally high levels of Cu and Fe in the brain catalyze the production of ROS, which further elicit oxidative stress contributing to the AD pathogenesis.[16] Thus, lowering the concentration of metals in the brain through chelation represents another rational therapeutic approach to treating AD.

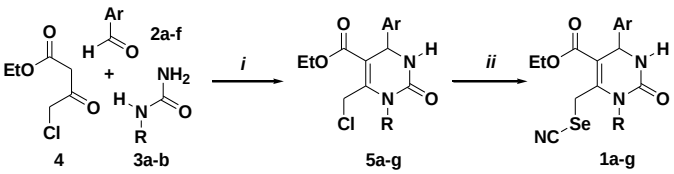
Drug candidates that target single processes have been advanced to the clinical stage, but they only provide relief of the symptoms and do not tackle the underlying causes of the disease, because of the multifactorial pathogenesis of neurodegenerative diseases. Modulation of multiple targets along the same biological pathway

could potentially lead to disease modification rather than just control of the symptoms[17] and the development of new drugs for AD is being focused on multipotent molecules acting in a complementary manner, which could be more efficacious to AD patients.[18] We are therefore interested in developing novel AChE inhibitors with antioxidant function for the treatment of AD by combining, in the same structure, biologically active dihydropyrimidinones (DHPMs) and organoselenium compounds (Figure 1). This strategy of merging two or more bioactive moieties in the same structure has been successfully employed in the development of new drug candidates.[19]
In this context, it is known that selenium (Se) might play different roles in the progression of AD.[20] In animal models of AD, Se has been shown to prevent oxidative damage and modulate the cholinergic system.[21] Organoselenium compounds, like diphenyl diselenide (Ph_2Se_2), can enhance the cognitive performance of rodents without inducing neurotoxicity,[22] while its *p*-methoxy analog (p-$MeOPh_2Se_2$) improves the memory of mice, protects against Aβ-induced neurotoxicity and inhibits the activity of AChE in the model of sporadic Alzheimer-type dementia, which can be explained by its antioxidant properties.[23] Moreover, organoselenium compounds have the ability to act as mimetics of the enzyme glutathione peroxidase (GPx),[24] which is known to have an important role in modulating oxidative stress in the brain.[25] Selenocyanides are metabolized to selenol,[26] being less toxic forms of organoselenium compounds[27] and have been employed successfully in the development of new bioactive compounds.[28]
DHPMs, readily obtained through the Biginelli reaction, are reportedly good antioxidants, acting as radical scavengers and being effective against lipid peroxidation,[29] with reports of some analogs being better radical scavengers than resveratrol.[30] The compounds in this class are potent AChE inhibitors,[31] with recent studies showing that the potency of some DHPM-curcuminoid hybrids is comparable to that of the standard drug galanthamine.[32]
Despite the advantages presented by DHPMs and organoselenium scaffolds in AD drug design, no studies have focused on designing multi-targeted hybrids of these structures within a single molecule. In this context, the aim of this study was to design a series of novel seleno-dihydropyrimidinones as potential multi-targeted therapeutics for AD, acting as antioxidants and AChE inhibitors. All compounds designed were synthesized and evaluated for their ability to inhibit AChE and to act as antioxidants through the glutathione peroxidase (GPx) mechanism, iron chelating activity, reducing power and total antioxidant capacity. Moreover, based on the molecule structure, the number of descriptors necessary to estimate the pharmacokinetics (PK) of the compounds was determined. As considerable effort went into the optimization of the pK of each substance at a very early stage of the research into its potential as a drug, the Lipinski and other drug-likeness filters were used to predict the pK of the compounds obtained.[33] Herein, we reported the design, synthesis, evaluation and predicted pK of a series of seleno-DHPM hybrids as potential multi-targeted therapeutics for AD.

Results and Discussion

Synthetic Procedures

The synthesis scheme involved a two-step pathway leading to the formation of a variety of seleno-dihydropyrimidinones **1a-g** in moderate yields (Scheme 1). A three-component Biginelli reaction aromatic aldehydes **2a-f**, urea **3a** or N-methyl-urea **3b** and ethyl 4-chloroacetoacetate **4** at 100°C, under neat conditions using HCl as the catalyst, produced the corresponding 6-chloromethyl-DHPMs **5a-g**.[34] These compounds were obtained in 68-99% yield after precipitation from water. The 6-chloromethyl-DHPMs obtained in this way were treated with potassium selenocyanide in MeOH at room temperature to afford the target hybrid selenocyanides **1a-g** in 22-43% yield after column chromatography. The structures of the compounds obtained are shown in Table 1. Their analytical and spectroscopic data are in agreement with the predicted structures. The seleno-DHPMs were found to be stable solids at room temperature, even when exposed to the atmosphere.



Scheme 1. *Reaction conditions. i)* $HCl_{conc.}$, neat, 100°C, 2h, 68-99%; *ii*) KSeCN (1.2 equiv.), MeOH, r.t., 24h.

Table 1. Structures and yields of the seleno-DHPMs

Entry	Ar	R	Compound	Yield (%)
1	C_6H_5-	H	**1a**	40
2	2-Me-C_6H_4-	H	**1b**	42
3	4-Me-C_6H_4-	H	**1c**	43
4	2-MeO-C_6H_4-	H	**1d**	42
5	4-MeO-C_6H_4-	H	**1e**	39
6	3-NO_2-C_6H_4-	H	**1f**	22
7	3-NO_2-C_6H_4-	Me	**1g**	32

In the ^{1}H NMR spectra the resonance signals of the N-H's are usually registered as *singlets* in the ranges of ca. 6.07-8.14 ppm and 9.42-9.67 ppm. The aromatic protons appear at between 6.85 and 8.05 ppm, and the chiral center at between 5.18 and 5.70 ppm as a *doublet*. The two protons attached to the selenide atom appear as two distinct *doublets* at around 3.96 to 4.34 ppm. A characteristic quartet and triplet, due to the ethyl ester moiety, can be found at around 4.05 and 1.05 ppm, respectively. The ^{13}C NMR spectra show the characteristic signal of the quaternary -CN carbon at between 100 and 103 ppm.
The IR spectra show a band in the region of approximately 2139-2154 cm^{-1}, corresponding to ν (C≡N). In the mass spectra (APPI), the molecular ion peaks M^+ presented the characteristic isotopic pattern of the organoselenium compounds.

Acethylcholinesterase inhibitory activity

All compounds considered were assessed as AChE inhibitors. The enzymatic activity was measured using an adaptation of the method described by Mata *et al.* (2007).[35] The concentrations of the test compounds that inhibited the hydrolysis of substrates by 50% (IC_{50} – Table 2) were determined by plotting the inhibition against the sample solution concentrations.

Table 2. Inhibitory activity of the seleno-DHPMs towards AChE.

Compound	AChE inhibition IC_{50} (ppm)	AChE inhibition IC_{50} (μmol.L^{-1})
1a	7.87	2.16
1b	2.44	8.58
1c	6.21	16.40
1d	6.11	15.49
1e	2.40	6.09
1f	7.72	18.87
1g	3.79	8.96
Galantamine	4.91	17.05

All compounds showed a high percentage inhibition of the enzyme acetylcholinesterase. The results demonstrate that all of the compounds were as active as the standard alkaloid galantamine, the active drug in Reminyl®, which is used in the treatment of Alzheimer's disease. The result obtained with the standard is in agreement with literature data.[36] No simple structure-activity relationship was observed for the compounds tested on comparing the electron donating or withdrawing substituents at different positions of the aromatic ring. Compound **1a**, bearing the simple phenyl substituent, is the most active of the series.

Antioxidant activity

There are several methods available for evaluating the *in vitro* antioxidant activity of biologically active substances, ranging from chemical assays with lipid substrates to more complex assays using many different instrumental techniques.[37] Due to the different types of free radicals and their different forms of action in living organisms, it is difficult to obtain a simple and universal method through which the antioxidant activity can be measured accurately and quantitatively. Thus, the search for more rapid and efficient tests has generated a great number of methods for the evaluation of the activity of antioxidants, through the use of a large variety of free radical generating systems.[38] Due to the wide divergence in the results of antioxidant tests, many protocols and guidelines have been established aimed at bringing order and agreement to this important field.[39]

In this study, the antioxidant activity of the seleno-DHPMs (Table 3) was investigated applying the following tests: reducing power, iron chelating activity, total antioxidant capacity and glutathione peroxidase assay (GPx). The results obtained are presented as mean ± SD.

Table 3. Antioxidant activity of seleno-DHPMs **1a-g**.

Compd.	GPx T_{50} (h)[a, b, c]	Iron chelating Activity (EC_{50} µg/mL)[d]	Reducing Power (mg AAE /g)[e]	Total antioxidant capacity (mg AAE/g)[e]
1a	4.92 ± 0.11	23.79± 0.14	2258.68 ± 23.54	521.81± 2.86
1b	5.55 ± 0.22	12.45± 0.03	2604.36 ± 12.37	638.17± 1.15
1c	5.04 ± 0.46	29.30± 0.26	2054.97± 11.75	461.74± 2.22
1d	6.70 ± 0.32	34.96± 0.17	1875.97 ± 3.26	391.42± 4.21
1e	5.07 ± 0.20	29.80± 0.11	2053.20± 13.44	457.61± 1.88
1f	6.04 ± 0.08	45.41± 0.33	1552.92 ± 8.65	287.88± 1.22
1g	6.15 ± 0.47	32.69± 0.22	1937.69± 11.00	416.36± 1.09
Ph_2Se_2	5.95 ± 0.47	----------	----------	----------
Rutin	----------	41,35± 0.16	1622.10 ± 11.76	310.43 ± 1.87
BHT	----------	20,65± 0.29	1641.89 ± 14.23	548.24 ± 1.23
EDTA	----------	4,65 ± 0.02	----------	----------

[a] Under these conditions addition of H_2O_2 in the absence of the organoselenium compound did not produce any significant oxidation of PhSH. [b] MeOH (1 mL); organoselenium catalyst (0.4 mM); PhSH (5 mM); H_2O_2 (10 mM). [c] T_{50} is the time required, in hours, to reduce the thiol concentration by 50% after the addition of H_2O_2; [d] EC_{50} value for 50% chelation of iron(II) ions; [e] Results, in mg, for ascorbic acid/ g of compound are calculated for sample concentrations of 100 µg/mL. Each value is expressed as mean ± SD.

We explored the potential antioxidant activity of all seleno-DHPMs **1a–g** using a GPx enzyme model based on the Tomoda method.[40] The reduction of H_2O_2 was monitored through the formation of diphenyl sulfide and increase in UV absorption at 305 nm. We measured the activity by considering the time required to reduce the thiol concentration by 50% (T_{50}) and used Ph_2Se_2 under the same conditions to compare the antioxidant activity.[41] The results obtained are summarized in Table 3. Encouragingly, all compounds showed activity levels comparable to that of PhSeSePh as GPx mimics. Compounds **1a-c** and **1e** provided the best results, being approximately 1.2 times more active than Ph_2Se_2. On the other hand, the hybrids **1d** and **1f-g** were less efficient as GPx mimics when compared to the standard. Although the seleno-DHPMs were assembled with substituents which have different electronic demands, their catalytic activity did not follow a clear trend and the effect (if any) of the substituent on their performance was negligible.

Reducing power is generally associated with the presence of reductones, which exert antioxidant action by breaking the free radical chain. In addition, reductones can reduce the oxidized intermediates of lipid peroxidation processes and thus act as primary and secondary antioxidants. The reducing power assay measures the electron donating ability of antioxidants using the potassium ferricyanide reduction method. Antioxidants cause the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form and activity is measured as the increase in the absorbance at 700 nm. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of the antioxidant samples.[42] The compounds analyzed showed excellent results for reducing power, probably due to the presence of the redox active selenocyanide moiety. All values were above those associated with the standards considered (rutin and BHT).

The antioxidant activity of compounds is also attributed to their ability to chelate transition metal ions, such as those of iron and copper, which have been proposed as catalysts for the initial formation of reactive oxygen species. Chelating agents may stabilize pro-oxidative metal ions in living systems through complexation.[43] Iron(II) ions are known to be a potent inducer of lipid peroxidation. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted resulting in a decrease in the red color of the complex. Measurement of the reduction in color intensity at the 562 nm wavelength allows an estimation of the metal chelating activity of the coexisting chelator.[44]

All compounds demonstrated the ability to chelate Fe^{2+}. Of the compounds analyzed, **1b** proved to be the most potent chelator, being more active than two standards and less potent than the most active of the standards (EDTA) by only a factor of 3. Of the seven compounds tested, six were more active than rutin. The values found for the standards are in agreement with literature data.[45] Importantly, the results for the reducing power and iron chelation showed a strong correlation (Adj.R-Square= 0.99851; y= -32.23369x + 3007.89974) indicating consistency in the values obtained applying to two tests.

Total antioxidant capacity was evaluated by the phosphomolybdenum method. This assay is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH with a maximum absorption at 695 nm.[46] The results obtained, expressed as ascorbic acid equivalents (AAE), are presented in Table 3. All samples investigated were active in a concentration-dependent manner and their potency values were high. Of the compounds tested **1b** showed the highest total antioxidant capacity with a value of 638.17 mg AAE per gram of compound. The antioxidant activity, assessed using this method, followed the same order observed in the previous tests, with strong correlations between reducing power and total antioxidant activity (Adj.R-Square 0.99897 and y= 0.33387x – 230.75251) and between iron chelating activity and total antioxidant capacity (Adj.R-Square 0.99886 and y= 0.33444x – 231.55161). All compounds essayed presented excellent results for antioxidant potential on applying a variety of methods, with very good correlations among the results obtained. In combination, these

findings indicate that the compounds have a high potential to act as antioxidants for the treatment of AD.

Preliminary Pharmacokinetics

To predict some aspects of the pharmacokinetics of the compounds obtained, their physicochemical and topological properties were calculated. Table 4 presents the octanol-water partition coefficients expressed as Clog *P* and Mlog *P* (Moriguchi log *P*), the number of H-Bond donors (HBD), H-Bond acceptors (HBA) and rotatable bonds (NRB), and the topological polar surface area (tPSA).[47]

Table 4. Molecular descriptors[a] *in silico* of seleno-DHPMs

Compd.	ClogP	MlogP	Mol. Weight	tPSA (Å^2)	HBA	HBD	Vol (Å^3)	NRB
1a	0.86	2.41	364.26	91.2	6	2	279.72	6
1b	1.17	2.81	378.29	91.2	6	2	296.28	6
1c	1.17	2.86	378.29	91.2	6	2	296.28	6
1d	0.75	2.42	394.29	100.5	7	2	305.26	7
1e	0.75	2.47	394.29	100.5	7	2	305.26	7
1f	0.59	2.34	409.26	137.1	9	2	303.05	7
1g	0.87	3.14	423.29	128.3	9	1	319.99	7

[a] tPSA, Topological polar surface area; HBA, H-bond acceptors; HBD, H-bond donors; NRB, Number of rotatable bonds.

The descriptors obtained *in silico* were compared with the filters for the prediction of the solubility and permeability of drug candidates after oral administration described by Lipinski,[48] Oprea[49] and Veber.[50] The results show that the compounds under consideration obey the Lipinski rule of 5: molecular weight (MW) ≤ 500, HBD ≤ 5, HBA ≤ 10 and log *P* ≤ 5.0 (Table 3). According to Lipinski, a compound whose data do not adhere to the rule will likely to be poorly bioavailable because of poor absorption or permeation.[48] The values for the compounds were MW 364.3-423.3 Da, HBD 1-2, HBA 6-9 and CLog *P* 0.59-1.17.

The seleno-DHPMs also satisfied Oprea's criteria, which additionally include: number of rings ≤ 5 and MLog *P* in the range of -2.0 and 4.5. The compounds possessed 2 rings and their MLog *P* values were in the range of 2.41-3.14. Veber proposed a filter of two properties: number of HBD and HBA ≤ 12 (tPSA ≤ 140 Å^2), and number of rotatable bonds (NRB) ≤ 10. All derivatives meet these criteria. It is postulated that limited molecular flexibility, expressed as the NRB, and low polar surface area (tPSA) are important predictors of oral bioavailability, independent of the molecular weight.

The results reported herein show that the compounds synthesized would have favorable pharmacokinetics on application, that is, solubility and permeability after the oral administration of drug candidates. They possess drug-likeness independent of the criterion used.

Experimental

General experimental procedures

All reactions were carried out under air atmosphere and monitored by TLC using Merck 60 F_{254} pre coated silica gel plates (0.25 mm thickness) and the products were visualized by UV detection, I_2 or Vanillin staining. Flash chromatography was carried out with silica gel (200-300 mesh). FT-IR spectra were recorded on a Varian 3100 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-*d*6 on a Bruker Avance 200 or Varian AS-400 spectrometer. Data for ^{1}H NMR are reported as chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, q = quartet, m = multiplet), coupling constant *J* (Hz), integration, and assignment, data for ^{13}C NMR are reported as a chemical shift (δ ppm). High resolution mass spectral analyses (HRMS) were carried out using APPI-Q-TOFMS measurements and were performed with a micrOTOF Q-II (Bruker Daltonics) mass spectrometer equipped with an automatic syringe pump (KD Scientific) for sample injection. The APPI-QTOF mass spectrometer was run at 4.5 kV with a desolvation temperature of 180 ºC. The mass spectrometer was operated in the positive ion mode. The standard atmospheric pressure photoionization (APPI) source was used to generate the ions. The sample was injected using a constant flow (3uL/min). The solvent was an acetonitrile/methanol mixture. The APPI-Q-TOF MS instrument was calibrated in the mass range of 50–3000 m/z using an internal calibration standard (low concentration tuning mix solution) supplied by Agilent Technologies. Data were processed employing Bruker Data Analysis software version 4.0. All the starting materials and catalysts were either purchased from commercial sources or synthesized by literature known procedures.[34] All the solvents were used without special treatment.

General procedure for the synthesis of seleno-DHPMs 1a-g

To a stirred solution of 6-chlorometyl-dihydropyrimidinone (3.0 mmol) in MeOH (10 mL) was added potassium selenocyanide (3.6 mmol). The resulting solution was stirred at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was extracted with ethyl acetate, water and brine respectively and dried with $MgSO_4$. The organic phase was evaporated by rotatory evaporator under reduced pressure to give the crude product. The crude product was purified by column chromatography over silica gel to furnish the pure product. The product was characterized by corresponding spectroscopic data (IR, ^{1}H and ^{13}CNMR, HRMS).

2-Oxo-4-phenyl-6-selenocyanatomethyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (1a): white solid, m.p. = 162^{o}C; IR (ν, cm^{-1}): 3396, 3219, 2149, 1703, 1675; ^{1}H NMR ($CDCl_3$, 200MHz): δ (ppm) 1.10 (t, J = 7.09 Hz, 3H), 4.01 - 4.11 (m, 4H), 5.18 (d, J = 2.69 Hz, 1H), 7.26 - 7.34 (m, 5H), 7.91 (s, 1H), 9.42 (s, 1H); ^{13}C NMR (DMSO-d6, 50MHz): δ (ppm) 13.8, 25.8, 53.9, 60.0, 100.4, 103.8, 126.4, 127.6, 128.5, 144.0, 148.3, 151.7, 165.2; HRMS (APPI-QTOF) m/z calcd. for $C_{15}H_{15}N_3O_3Se$ [M+H]: 366.0352; found 366.0354.

2-Oxo-6-selenocyanatomethyl-4-o-tolyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (1b): slightly yellow solid, m.p. = 178^{o}C; IR (ν, cm^{-1}): 3343, 3219, 2150, 1706, 1670; ^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 1.02 (t, J = 7.03 Hz, 3H), 2.39 (s, 3H), 3.96 – 4.11 (m, 4H), 5.61 (s, 1H), 6.57 (s, 1H), 7.10 – 7.21 (m, 3H), 7.29 (d, J = 7.42 Hz, 1H); 9.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 100MHz): δ (ppm) 13.7, 18.8, 26.7, 51.5, 60.6, 101.2, 103.7, 126.8, 127.3, 127.9, 130.5, 134.7, 140.8, 147.3, 152.8, 165.6; HRMS (APPI-QTOF) calcd. for $C_{16}H_{17}N_3O_3Se$ [M+H]: 380.0509; found 380.0512.

2-Oxo-6-selenocyanatomethyl-4-p-tolyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (1c): slightly yellow solid, m.p. = 152^{o}C; IR (ν, cm^{-1}): 3342, 2153, 1730, 1675; ^{1}H NMR ($CDCl_3$, 200MHz): δ (ppm) 1.14 (t, J = 7.07 Hz, 3H), 2.97 (s, 3H),

3.97 – 4.12 (m, 4H), 5.32 (d, J = 3.03 Hz, 1H), 6.67 (s, 1H), 7.10 (d, J = 8.59 Hz, 2H), 7.20 (d, J = 8.59 Hz, 2H), 9.55 (s, 1H); ^{13}C NMR (DMSO-d6, 50MHz): δ (ppm) 13.8, 20.9, 26.6, 54.7, 60.6, 101.8, 103.5, 126.5, 129.25, 137.7, 139.9, 146.6, 153.5, 165.5; HRMS (APPI-QTOF) calcd. for $C_{16}H_{17}N_3O_3Se$ [M+H]: 380.0509; found 380.0510.

4-(2-Methoxy-phenyl)-2-oxo-6-selenocyanatomethyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (1d): slightly yellow solid, m.p. = 140°C; IR (ν, cm^{-1}): 3359, 3225, 2154, 1714, 1677; ^{1}H NMR ($CDCl_3$, 200MHz): δ (ppm) 1.07 (t, J = 7.34 Hz, 3H), 3.86 (s, 3H), 3.99 – 4.08 (m, 3H), 4.22 (d, J = 12.23 Hz, 1H), 5.70 (d, J = 2.45 Hz, 1H), 6.07 (s, 1H), 6.87 – 6.93 (m, 2H), 7.10 (d, J = 5.87 Hz, 1H), 7.27 (t, J = 7.83 Hz, 1H), 9.58 (s, 1H); ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 13.9, 26.8, 50.5, 55.3, 60.6, 99.3, 103.2, 110.7, 120.5, 127.1, 129.2, 129.4, 148.3, 153.6, 156.9, 165.8; HRMS (APPI-QTOF) calcd. for $C_{16}H_{17}N_3O_4Se$ [M+H]: 396.0458; found 396.0461.

4-(4-Methoxy-phenyl)-2-oxo-6-selenocyanatomethyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (1e): slightly yellow solid, m.p. = 155°C; IR (ν, cm^{-1}): 3353, 2153, 1728, 1672; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.15 (t, J = 7.03 Hz, 3H), 3.78 (s, 3H), 4.03 – 4.09 (m, 4H), 5.32 (s, 1H), 6.26 (s, 1H), 6.85 (d, J = 8.60 Hz, 2H), 7.23 (d, J = 8.60 Hz, 2H), 9.55 (s, 1H); ^{13}C NMR (DMSO-d6, 100MHz): δ (ppm) 13.9, 25.9, 53.3, 55.1, 60.0, 100.6, 103.9, 113.8, 127.6, 136.3, 148.1, 151.8, 158.7, 165.3; HRMS (APPI-QTOF) calcd. for $C_{16}H_{17}N_3O_4Se$ [M+H]: 396.0458; found 396.0457.

4-(3-Nitro-phenyl)-2-oxo-6-selenocyanatomethyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (1f): slightly yellow solid, m.p. = 166°C; IR (ν, cm^{-1}): 3427, 3320, 2151, 1722, 1684; ^{1}H NMR (DMSO-d6, 200 MHz): δ (ppm) 1.17(t, J=7.07HZ, 3H), 3.96 – 4.07 (m, 3H), 4.23 (d, J = 12,13 Hz, 1H), 5.38 (d, J = 3,03 Hz, 1H), 7.42 – 7.51 (m, 2H), 7.65 (m, 1H), 8.05-8.14 (m, 2H), 8.14 (s, 1H), 9.67 (s, 1H); ^{13}C NMR (DMSO-d6, 100MHz): δ (ppm) 13.6, 30.4, 53.4, 60.0, 99.3, 102.8, 121.2, 122.2, 129.7, 132.7, 146.0, 147.7, 149.1, 151.4, 164.7; HRMS (APPI-QTOF) calcd. for $C_{15}H_{14}N_4O_5Se$ [M+H]: 411.0203; found 411.0208.

1-Methyl-4-(3-Nitro-phenyl)-2-oxo-6-selenocyanatomethyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (1g): slightly yellow solid, m.p. = 173°C; IR (ν, cm^{-1}): 3432, 3263, 2139, 1699, 1673; ^{1}H NMR (DMSO-d6, 200 MHz): δ (ppm) 1.14 (t, J = 7.09 Hz, 3H), 3.30 (s, 3H), 4.07 (q, J = 7.09 Hz, 2H), 4.33 (s, 2H), 5.39 (d, J = 3.91 Hz, 1H), 7.42 – 7.61 (m, 2H), 7.95 (d, J = 3.67 Hz, 1H), 8.03 – 8.13 (m, 2H); ^{13}C NMR (DMSO-d6, 100MHz): δ (ppm) 13.7, 24.6, 29.5, 51.6, 60.4, 103.2, 103.8, 121.2, 122.3, 129.7, 132.5, 145.2, 147.7, 150.1, 152.6, 164.9; HRMS (APPI-QTOF) calcd. for $C_{16}H_{16}N_4O_5Se$ [M+H]: 425.0359; found 425.0364.

Acetylcholinesterase activity

The enzymatic activity was measured using an adaptation of the method described by Mata *et al* (2007). Briefly, 300 μL of 50 mmol L^{-1} Tris-HCl buffer, pH 8.0, 100 μL of a buffer solution containing the sample at five different concentrations dissolved in MeOH and 50 L μof an AChE solution containing 0.28 U mL^{-1} (50 mmol L^{-1} Tris-HCl, pH 8.0 buffer, 0.1% BSA) were incubated for 15 min. Then, 75 μL of an acetylthiocholine iodide solution (0.023 mg mL^{-1} in water) and 475 μL DTNB (3 mmol L^{-1} in Tris-HCl, pH 8.0 buffer, 0.1 mol L^{-1} NaCl, 0.02 mol L^{-1} $MgCl_2$) were added, and the final mixture was incubated for another 30 min at room temperature. The absorbance of the mixture was measured at 405 nm. A control mixture containing methanol instead of the sample was considered to have 100% AChE activity. The inhibition (%) was calculated as follows: I (%) = 100 – (Asample/Acontrol) × 100 in which Asample is the absorbance of the sample and Acontrol is the absorbance without sample. The tests were performed in triplicate, and a blank containing Tris-HCl buffer was used instead of the enzyme solution. The sample concentration with 50% inhibition (IC_{50}) was determined by plotting the inhibition against the sample solution concentrations. The galantamine was used as the positive control.

Total antioxidant capacity

Total antioxidant capacities of compounds were evaluated by phosphomolybdenum method. This assay is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH with a maximal absorption at 695 nm.[46]

Reduction power

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action. The reducing power of compounds was determined according to the method of Yen and Chen (1995). Different amounts of each compounds (25 - 250μgml^{-1}) in methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Iron chelating activity

Solutions of compounds (1 mL) in different concentrations were evenly mixed with 0.05 mL $FeCl_2$ (2 mM), and added with 0.2 mL ferrozine solution (5 mM). The mixtures were shaken and left standing at room temperature for 20 min, the absorbance values (Asample) of the mixtures were measured at 562 nm. Metanol was used instead of the sample solution as blank control (Ablank) and Na_2EDTA was used as positive control. Fe^{2+} chelating rate (%) = 100 × [(*A*blank - *A*sample) / *A*blank].[51]

Determination of GPx-like activity

The experiments were made according to the Tomoda method.[40] The catalytic GPx model reaction was initiated by the addition of H_2O_2 (final concentration: 10 mM) to a methanol solution (final volume: 1 mL) of thiophenol (PhSH) (final concentration 5 mM) containing the selenium catalyst (final concentration: 0.4 mM) at 25 (±3) ° C. The formation of PhSSPh was monitored by UV spectrophotometry, at 305 nm. Absorbance-versus time data were

stored directly on a microcomputer. The reaction was followed for 6 minutes and three times under the same conditions.

Conclusions

In summary, we designed, synthesized and evaluated a series of novel seleno-DHPMs as potential multi-target therapeutics for Alzheimer's disease. The compounds showed excellent activity as inhibitors of AChE, all being more active than the standard drug. All compounds were essayed and showed very good antioxidant activity through different mechanisms of action. Their GPx mimetic activity, iron chelating activity, reducing power and total antioxidant activity were demonstrated. Moreover, some pharmacokinetics parameters were calculated and all of the seleno-DHPMs investigated would have favourable pharmacokinetics on application and possess drug-like properties.

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Notes and references

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1 *(a)* G. Stix, *Sci. Am.* 2010**,** **302**, 50; *(b)* World Health Organization, Facts and figures of Alzheimer's disease: the brain killer, 2012.

2 F. Belluti, L. Piazzi, A. Bisi, S. Gobbi, M. Bartolini, A. Cavalli, P. Valenti and A. Rampa, *Eur. J. Med. Chem.* 2009, **44**, 1341.

3 *(a)* F. Leonetti, M. Catto, O. Nicolotti, L. Pisani, A. Cappa, A. Stefanachi and A. Carotti, *Bioorg. Med. Chem.* 2008, **16**, 7450; *(b)* S. Akasofu, M. Kimura, I. Kosasa, K. Sawada and H. Ogura, *Chem. Biol. Interact.* 2008, **175**, 222; *(c)* R. Sheng, X. Lin, J. Y. Li, Y. K. Jiang, Z. C. Shang and Y. Z. Hu, *Bioorg. Med. Chem. Lett.* 2005, **15**, 3834.

4 P. Camps, X. Formosa, C. Galdeano, T. Gomez, D. Munoz-Torrero, L. Ramirez, E. Viayna, E. Gomez, N. Isambert, R. Lavilla, A. Badia, M. V. Clos, M. Bartolini, F. Mancini, V. Andrisano, A. Bidon-Chanal, O. Huertas, T. Dafni and F. J. Luque, *Chem. Biol. Interact*. 2010, **187**, 411.

5 R. Hoerr and M. Noeldner, *CNS Drug Rev.* 2002, **8**, 143.

6 H. Sugimoto, Y. Yamanishi, Y. Iimura and Y. Kawakami, *Curr. Med. Chem.* 2000, **7**, 303.

7 S. Kavanagh, B. Gaudig, B. V. Baelen, M. Adami, A. Delgado, C. Guzman, E. Jedenius and B. Schaeuble, *Acta Neurol. Scand.* 2011, **124**, 302.

8 H. Soreq and S. Seidman, *Nat. Rev. Neurosci.* 2001, **2**, 294-302; P. Jia, R. J. Sheng, L. Zhang, Q. Fang, B. He and Y. Yang, *Eur. J. Med. Chem.* 2009, **44**, 772.

9 L. Pan, J.-H. Tan, J.-Q. Hou, S.-L. Huang, L.-Q. Gu and Z.-S. Huang, *Bioorg. Med. Chem. Lett.* 2008, **18**, 3790; M. Bartolini, C. Bertucci, V. Cavrini and V. Andrisano, *Biochem. Pharmacol.* 2003, **65**, 407.

10 M. A. Lovell, S. Xiong, G. Lyubartseva and W. R. Markesbery, *Free Rad. Biol. Med.* 2009, **46**, 1527.

11 W. R. Markesbery and M. A. Lovell, *Arch. Neurol.* 2007, **64**, 954.

12 Y. Christen, *Am. J. Clin. Nutr.* 2000, **71**, 621s.

13 *(a)* A. I. Bush and R. E. Tanzi, *Neurotherapeutics* 2008, **5**, 421; *(b)* K. Jomova, D. Vondrakova, M. Lawson and M. Valko, *Mol. Cell Biochem.* 2010, **345**, 91.

14 P. Zatta, D. Drago, S. Bolognin and S. L. Sensi, *Trends Pharmacol. Sci.* 2009, **30**, 346.

15 A. I. Bush, *J. Alzheimer's Dis.* 2008, **15**, 223.

16 H. Zheng, M. B. Youdim and M. Fridkin, *J. Med. Chem.* 2009, **52**, 4095.

17 P. C. Trippier, K. J. Labby, D. D. Hawker, J. J. Mataka and R. B. Silverman, *J. Med. Chem.* 2013, **56**, 3121.

18 *(a)* J. Marco-Contelles, R. Léon, M. G. López, A. G. García and M. Villarroya, *Eur. J. Med. Chem.* 2006, **41**, 1464; *(b)* L. Fang, B. Kraus, J. Lehmann, J. Heilmann, Y. Zhang and M. Decker, *Bioorg. Med. Chem. Lett.* 2008, **18**, 2905; *(c)* W. J. Geldenhuys, M. B. H. Youdim, R. T. Carroll and C. J. Van der Schyf, *Progress in neurobiol.* 2011, **94**, 347.

19 C. A. M Fraga, *Expert Opin. Drug Dis.* 2009, 4, 605; B. Meunier, *Acc. Chem. Res.* 2008, 41, 69

20 M. Loef, G. N. Schrauzer and H. Walach, *J. Alzheimers Dis.* 2011, **26**, 81; U. Cornelli, *Neurodegener. Dis.* 2010, **7**, 193.

21 A. C. Souza, C. A. Brüning, M. R. Leite, G. Zeni and C. W. Nogueira, *Behav. Pharmacol.* 2010, **21**, 556.

22 *(a)* R. M. Rosa, D. G. Flores, H. R. Appelt, A. L. Braga, J. A. P. Henriques and R. Roesler, *Neurosci. Lett.* 2003, **341**, 217; *(b)* E. C. Stangherlin, C. Luchese, S. Pinton, J. B. Rocha and C. W. Nogueira, *Brain Res.* 2008, **1201**, 106.

23 *(a)* S. Pinton, J. T. Rocha, B. M. Gai, M. Prigol, L. V. Rosa and C. W. Nogueira, *Cell Biochem. Funct.* 2011, **29**, 235; *(b)* S. Pinton, J. T. Rocha, G. Zeni and C. W. Nogueira, *Neurosci. Lett.* 2010, **472**, 56; *(c)* S. Pinton, C. A. Brüning, C. E. S. Oliveira, C. Prigol and C. W. Nogueira, *J. Nutrit. Biochem.* 2013, **24**, 311; *(d)* S. Pinton, A. C. Souza, M. H. M. Sari, R. M. Ramalho, C. M. P. Rodrigues and C. W. Nogueira, *Behav. Brain Res.* 2013, **247**, 241.

24 *(a)* K. P. Bhabak and G. Mugesh, *Acc. Chem. Res.* 2010, **43**, 1408; *(b)* N. M. R. McNeil, M. C. Matz and T. G. Back, *J. Org. Chem.*, 2013, **78**, 10369; *(c)* E. E. Alberto, V. Nascimento, A. L. Braga, *J Brazil Chem Soc*, 2010, **21,** 2032; *(d)* C. T. Santi, C. Scalera, M. Piroddi and F. Galli, *Curr. Chem. Biol.*, 2013, **7,** 25; *(e)* F. Kumakura, B. Mishra, K. I. Priyadarsini and M. Iwaoka, *Euro. J. Org. Chem.*, 2010, **3**, 440; *(f)* A.L. Braga and J. Rafique (2013) 'Synthesis of biologically relevant small molecules containing selenium. Part A. Antioxidant compounds', in *The Chemistry of Organic Selenium and Tellurium Compounds, Volume 4*, edited by Z. Rappoport. John Wiley & Sons, Ltd: Chichester, UK, pp. 989-1052. *(g)* V. Nascimento, E. E. Alberto, D. W. Tondo, D. Dambrowski, M. R. Detty, F. Nome and A. L. Braga, *J. Am. Chem. Soc.*, 2012, **134**, 138.

25 P. Klivenyi, O. A. Andreassen, R. J. Ferrante, A. Dedeoglu, G. Mueller and E. Lancelot, *J. Neurosci.* 2000, **20**, 1.
26 I. Clement, *J. Nutr*. 1998, 128, 1845
27 S. S. Roy, P. Ghosh, U. H. Sk, P. Chrakaborty, J. Biswas, S. Mandal, A. Bhattacharjee and S. Bhattacharya, *Bioorg. Med. Chem. Lett.* 2010, 20, 6951
28 S. U. Hossain, S. Sengupta and S. Bhattacharia, *Bioorg. Med. Chem.* 2005, 13, 5750; G. Krishnegowda, A. S. P. Gowda, H. R. S. Tagaram, K. F. S. Carroll, R. B. Irby, A. K. Sharma and S. Amin, *Bioorg. Med. Chem.* 2011, 19, 6006; D. Plano, Y. Baquedano, D. Moreno-Mateos, M. Font, A. Jiménez-Ruiz, J. A. Palop and C. Sanmartín, *Eur. J. Med. Chem.* 2011, 46, 3315; U. H. Sk, A. K. Sharma, S. Ghosh and S. Bhattacharya, *Eur. J. Med. Chem.* 2010, 45, 3265
29 *(a)* B. R. P. Kumar, G. Sankar, R. B. N. Baig and S. Chandrashekaran, *Eur. J. Med. Chem.* 2009, **44**, 4192; *(b)* A. Vasconcelos, P. S. Oliveira, M. Ritter, R. A. Freitag, R. L. Romano, F. H. Quina, L. Pizzuti, C. M. P. Pereira, F. M. Stefanello and A. G. Barschak, *J. Biochem. Mol. Toxicol.* 2012, **26**, 155; *(c)* H. A. Stefani, C. B. Oliveira, R. B. Almeida, C. M. P. Pereira, R. C. Braga, R. Cella, V. C. Borges, L. Savegnago and C. W. Nogueira, *Eur. J. Med. Chem.* 2006, **41**, 513.
30 D. L. Silva, F. S. Reis, D. R. Muniz, A. L. T. G. Ruiz, J. E. Carvalho, A. A. Sabino, L. V. Modolo and A. Fátima, *Bioorg. Med. Chem.* 2012, **20**, 2645.
31 *(a)* I. Tomassoli, L. Ismaili, M. Pudlo, C. de los Ríos, E. Soriano, I. Colmena, L. Gandía, L. Rivas, A. Samadi, J. Marco-Contelles and B. Refouvelet, *Eur. J. Med. Chem.* 2011, **46**, 1; *(b)* H. Zhi, C. Zhang, Z. Cheng, Z. Jin, E. Huang, S. Li, H. Lin, D. C. Wan and C. Hu, *Med. Chem.* 2013, **9**, 703.
32 S. Arunkhamkaew, A. Athipornchai, N. Apiratikul and A. Suksamrarn, *Bioorg. Med. Chem. Lett.* 2013, **23**, 2880.
33 *(a)* C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Deliv. Rev.* 1997, **23**, 3; *(b)* C. A. Lipinski, *J. Pharmacol. Toxicol. Methods* 2000, **44**, 235; *(c)* D. F. Veber, S. R. Johnson, H. Y. Cheng, B. R. Smith, K. W. Ward and K. D. Kopple, *J. Med. Chem.* 2002, **45**, 2615.
34 *(a)* H. Sharghi and M. Jokar, *Synth. Commun.* 2009, **39**, 958; *(b)* P. S. Lebed, P. O. Kos, V. V. Polovinko, A. A. Tolmachev and M. V. Vovk, *Russ. J. Org. Chem.* 2009, **45**, 921.
35 A.T. Mata, C. Proença, A. R. Ferreira, M. L. M. Serralheiro, J. M. F. Nogueira and M. E. M. Araújo, *Food Chem.* 2007, **103**, 778.
36 I. Ahmad, I. Anis, A. Malik, S. A. Nawaz and M. I. Choudhary *Chem. Pharm. Bull.* 2003, **51**, 412.
37 C. Sánchez-Moreno *Food Sci. Tech. Int.* 2002, **8**, 121.
38 C. Q. Alves, J. M. David, J. P. David, M. V. Bahia and R, M, Aguiar *Quím. Nova* 2010, **33**, 2202.
39 E. D. Frankel and J. W. Finley *J. Agric. Food Chem.* 2008, **56**, 4901.
40 M. Iwaoka and S. Tomoda, J. Am. Chem. Soc. 1994, **116**, 2557.
41 L. C. Soares, E. E. Alberto, R. S. Schwab, P. S. Taube, V. Nascimento, O. E. Rodrigues and A. L. Braga *Org. Biomol.Chem.* 2012, **10**, 6595
42 T. Ak and İ. Gülçin *Chem. Biol. Interact.* 2008, **174**, 27; K. N. Prasad, B. Yang, X. Dong, G. Jiang, H. Zhang, H. Xie and Y. Jiang *Innov. Food Sci. Emerg. Technol.* 2009, **10**, 627.
43 F. Shahidi, C. M. Liyana-Pathirana and D.S. Wall *Food Chem.* 2006, **99**, 478.
44 İ Gülçin *Toxicology* 2006, **217**, 213.
45 S. Vladimir-Knezevic, B. Blazekovic, M. B. Stefan, A. Alegro, T. Koszegi and J. Petrik *Molecules* 2011, **16**, 1454.
46 P. Prieto, M. Pineda and M. Aguilar *Anal. Biochem.* 1999, **269**, 337.
47 P. Ertl, B. Rohde and P. Selzer, *J. Med. Chem.* 2000, **43**, 3714.
48 *(a)* C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney *Adv. Drug Deliv. Rev.* 1997, **23**, 3; *(b)* C. A. Lipinski *J. Pharmacol. Toxicol. Methods* 2000, **44**, 235.
49 T. I. Oprea, J. Gottfries, V. Sherbukhin, V. Svensson and T. C. Kuhler *J. Mol. Graph. Mod.* 2000, **18**, 512.
50 D. F. Veber, S. R. Johnson, H. Y. Cheng, B. R. Smith, K. W. Ward and K. D. Kopple *J. Med. Chem.* 2002, **45**, 2615.
51 J. Wang, Q. Zhang, Z. Zhang and Z. Li, *Int. J. Biol. Macromol.* 2008, **42**, 127.