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Review Article

Direct- And Spacer-Coupled Codrug Strategies for the Treatment of Alzheimer's Disease

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Abstract

Over the last years, the 'multi-target-directed ligand' strategy has been exploited by many researchers to develop novel attractive tools in the search for new agents for Alzheimer's disease (AD). Small molecules that concurrently target and modulate AD multiple pathological factors can be synthesized using such strategy. This paper will mainly focus on direct- and spacer-coupled codrug approaches that we recently rationally used to design multifunctional molecules able to contrast oxidative stress, neuroinflammation, glutamate toxicity, and metal dyshomeostasis, as a function of the structural elements introduced in the chemical framework.

Although the potential use of these strategies needs further exhaustive studies, it may offer a promising therapeutic alternative for increasing neuronal protection and preventing AD progression.

Keywords: Alzheimer's disease; Multi target direct ligand strategy; Codrug; Glutathione; Lipoic acid

Introduction

Alzheimer's disease (AD) is a disabling brain disorder that is going to involve 100 million people all over the world by 2050 [1]. AD is marked by gradual memory loss and cognitive decline connected to a decrement of cholinergic neurons and acetylcholine (AChE) levels. Neuritic plaques – composed of amyloid- β protein (Aβ) – and Neurofibrillary Tangles (NFT) – formed by hyperphosphorylated tau proteins (τ) – characterize AD affected brain [2]. Among different isoforms produced by the consecutive hydrolysis of the Aβ precursor (AβPP) mediated by β- and γ-secretases [3,4], the $A\beta_{1-42}$ isoform in the brain intensifies the tendency for peptide aggregation [5] leading to a quickened formation of small Aβ oligomers [6,7]. In addition, after hyperphosphorylation, τ-protein detaches from the microtubules and forms NFT intracellular neurotoxic aggregates [8]. These last, together with Aβ oligomers, are connected to neurotoxicity in AD brains.

The etiology of AD still remains obscure, but several events – for instance oxidative stress, Aβ plaques, metal dyshomeostasis, excitotoxicity, protein misfolding, and inflammatory processes – play a strategic role in the advancement of AD [9,10]. It is intricate to found the right succession of these events, but it was proved that oxidative damage is one of the initial pathological markers [11-15]. Oxidative stress arises when the normal equilibrium between oxidative events and antioxidant defenses is interrupted either by deficiency of antioxidant enzymes or by incremented production of Reactive Oxygen Species (ROS).

In these pathological conditions, ROS react with lipids, proteins, nucleic acids, and other molecules thus altering their structure and function [16]. ROS generation in AD brains could be produced by high amounts of metal ions (Fe, Al, and Hg) or by Aβ aggregates [17]. In fact, the incubation of Cu(II), Zn(II), or Fe(III) with Aβ oligomers promotes the development of $A\beta_{1-40}$ and $A\beta_{1-42}$ deposits; notably, the deposition of fibrillar amyloid plaques is enhanced by Fe(III), while the formation of amorphous aggregates is induced by Cu(II) and Zn(II) [18]. Besides, Aβ plaques are responsible for inflammation in AD brains: *in vitro* studies on astrocytes have convincingly demonstrated that Aβ and APP activate glia in a dose- and time-dependent manner, as measured by morphological response and expression of potent pro-inflammatory cytokines and TNF–α [19]. Neuroinflammatory processes that characterize AD might also be responsible for the depletion of Glutathione (GSH), the main tripetide able to counteract the oxidant injury [20,21]; effectively, some researchers found lowered GSH levels in AD brains [22,23]. Altogether, these data confirm that reduced antioxidant systems, metal ion dyshomeostasis, neuroinflammation, and Aβ aggregation generate a vicious circle closely related to the onset of AD.

Multi-Target Therapy for the Cure of AD

Current cure for AD focuses on symptomatic aspects of the pathology and four cholinesterase inhibitors (tacrine, donepezil, rivastigmine, and galantamine) and Memantine (MEM) are the only drugs approved by FDA. Acetylcholinesterase inhibitors enhance cholinergic neurotransmission through inhibition of acetylcholinesterase, thus decreasing the breakdown of ACh [24,25]. MEM - an uncompetitive N-methyl-D-aspartate (NMDA) antagonist - is the only drug employed in the therapy of moderate to severe dementia [26,27]. Nevertheless, all these drugs are not satisfactory to heal AD or to stop its progression. In fact, as AD is featured by a multifactorial nature, drugs are required to hit the pathology at different levels simultaneously [28].

In recent years, several medicinal chemistry approaches were proposed in the search for novel drug candidates [29]. Among them, the 'Multi-Target-Directed Ligand' (MTDL) strategy [30-33]

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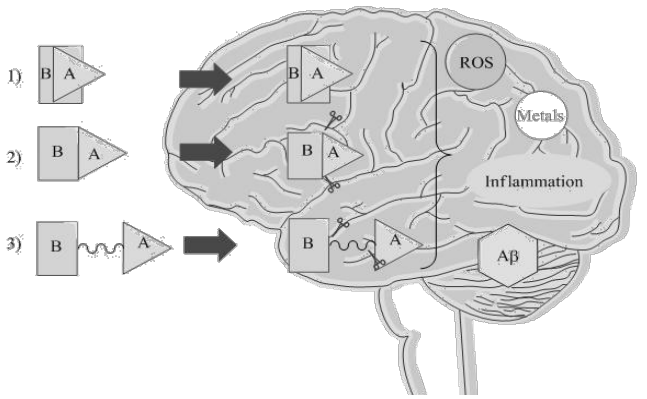


Figure 1: Schematic representation of MTDL strategy. 1) Hybrid molecule consists of two or more pharmacophores (A and B) fused to each other that do not require enzymatic activation to exert the biological effects; 2) Direct-coupled codrug consists of two or more pharmacophores (A and B) linked to each other via a covalent bond that explicate the biological effects after enzymatic biotransformation; 3) Spacer-coupled codrug consists of two or more pharmacophores (A and B) linked to each other via a linker that is cleavable after enzymatic activity, releasing individually A and B able to operate at the active sites.

is drawing attention of many researchers (Figure 1). Indeed, small molecules that concurrently target and modulate the AD pathological factors can be designed using this methodology. Hybrids and codrugs are examples of MTDLs [34]: the first ones are constituted by two diverse pharmacophores joined via a permanent bond, and that exert a dual effect resulting from a single chemical entity acting on different biological targets without undergoing enzymatic cleavage; on the other hand, codrugs consist of two or more pharmacophores direct-and/or spacer-coupled via a covalent chemical linkage that, after enzymatic biotransformation, explicate their biological effects. The major limitation of this approach is the requirement of well-designed groups for the linker [34], whereas the main advantage is represented by the advancement of pharmacokinetic profiles of the single drugs. Figure 1 reports a schematic representation regarding the different ways of action of both hybrids and codrugs. Many examples of hybrids were proposed for the cure of AD and the most successful ones were reported in Table 1 [36-46].

The codrug strategy has been widely used for the discovery of novel compounds in many therapeutic fields (i.e. anticancer, antibacterial, antiparkinson, antiviral), whereas it was less explored to discover novel MTDLs for AD [34].

Recently, Chen *et al.* developed the tacrine-silibinin codrug (**16**), obtained through conjugation of tacrine to silibinin, a natural compound provided with neuroprotective properties (Figure 2) [35]. This codrug, in addition to the decrease of epatotoxicity due to tacrine, resulted endowed with potent AChE and BuChE inhibition properties (IC_{50} = 53.9 and 49.7 nM, respectively).

Because of the lack of literature data regarding AD codrugs, the present work is intended as an overview which mainly focuses on codrugs that we synthesized to simultaneously strengthen neuronal protection and avoid AD progression (Figure 3).

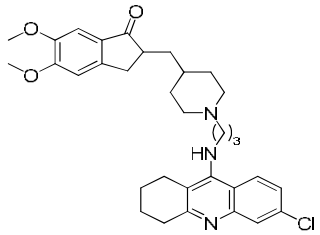
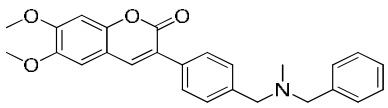
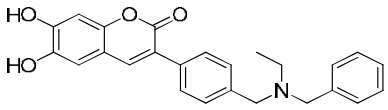
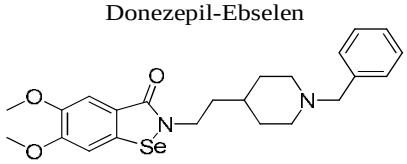
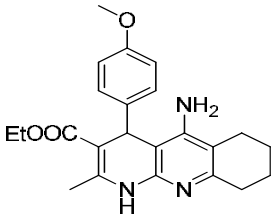
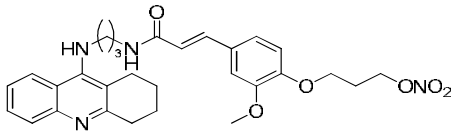
Ibuprofen-Glutathione, Ibuprofen-Lipoic Acid and Lipoic acid-GPE Codrugs against Oxidative Stress and Neuroinflammation in AD

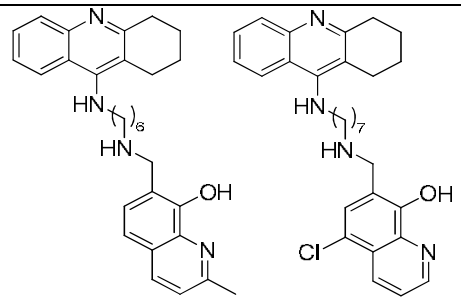
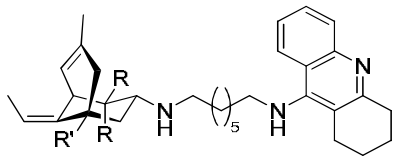
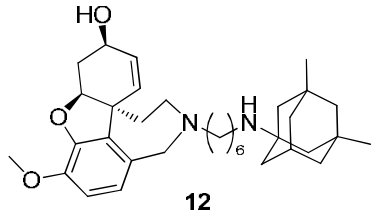
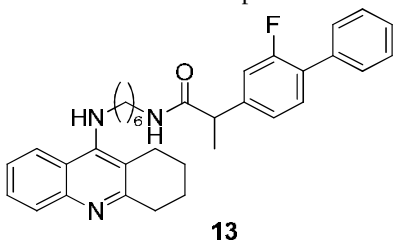
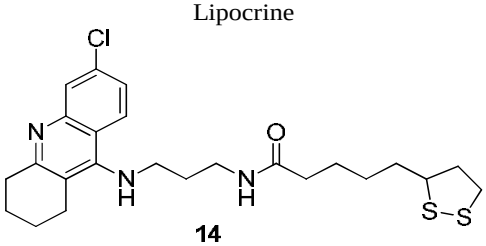
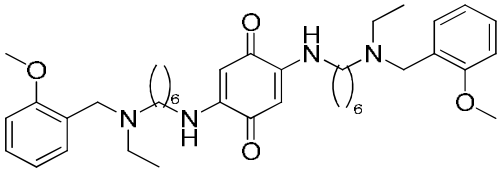
Neuroprotective antioxidants constitute a promising class of molecules for the treatment of AD [47-50]. Among them, sulfur-containing compounds [GSH, lipoic acid (LA), and other thiol derivatives] are able to hamper cell damage by counteracting ROS through different modes of action [51]. In this context, GSH behaves as direct or indirect scavenger of ROS in brain cells and works as substrate for enzymes implicated in detoxification processes [52]. GSH depletion is well documented in many neurodegenerative disorders, hence its restoration might be a potential strategy for the management of such pathologies. The uptake of GSH through the Blood-Brain-Barrier (BBB) is regulated by its membrane transporters; in fact, these last are capable of recognizing the GSH portion even when bound to other molecules which act as shuttles for the delivery of drugs inside the brain [53]. Alternatively, GSH levels can be reverted using GSH prodrugs, codrugs, and analogs, and cysteine derivatives. On the basis of our interest for short bioactive peptides, in 2007 we started to synthesize novel GSH-containing codrugs for the treatment of neurodegenerative diseases. Therefore, the direct-coupled codrug strategy was employed for the conjugation of ibuprofen to GSH (IBU-GSH, **17**) via an amide bond (Figure 3) [54]. AD affected patients found melioration following the treatment with IBU, as it specially reduces the α1-antichymotrypsin-mediated amyloidogenesis [55] and $A\beta_{1-42}$ production, thus preventing the advancement of AD [56,57] in virtue of an allosteric modulation of γ-secretase activity. Due to the conjugation with GSH, IBU is dragged into the BBB explicating its antinflammatory activity directly on impaired neurons. Evaluation of IBU-GSH physico-chemical properties suggested that codrug **17** could be orally administered since it showed water solubility higher than 10 μg/mL and LogP value superior to 1.35 (Table 2); moreover, this LogP value is also appropriate for CNS penetration. Kinetic data revealed a good stability in simulated fluids (pH 1.3 and 7.4, $t_{1/2}$ > 16 h), while in human plasma a slow bioconversion to IBU was observed ($t_{1/2}$ > 0.7 h) (Table 3).

Apart from its ROS scavenging activity, IBU-GSH was also assayed for its ability to antagonize the harmful effects of $A\beta_{1-40}$ in an *in vivo* experimental model. In behavioral tests of long-term spatial memory, rats treated with codrug **17** (23 μmol/Kg for 28 days) showed a significant improvement compared to the controls (rat treated only with $A\beta_{1-40}$, IBU + $A\beta_{1-40,}$ GSH + $A\beta_{1-40}$, respectively). Behavioral results were strengthened by histochemical analysis, showing a lower expression of Aβ plaques and pyknotic cells in Aβ-infused rat cerebral cortex [54,58].

In the two-year period 2010-2012, we synthesized other codrugs containing IBU conjugated to LA via an amide bond (IBU-LA, **18-20**) (Figure 3) using the spacer-coupled codrug strategy [59-62]. LA has proved to be a ROS scavenger, a chelant of metals, a GSH precursor [63] and a good BBB-crossing agent [64]. For the synthesis of codrugs **18-20** we selected polyamine chains of different length as chemical spacers, as they are involved in many signaling pathways regulating the expression of different proteins [65]. Oral administration of codrugs **18-20** could be suitable since their water solubility is higher than 10 μg/mL and LogP values are superior to 1.35 (Table 2); in addition, cLogP values (> 4) favor their BBB

Table 1: Recent examples of the most promising hybrids proposed as MTDLs for the treatment of AD.

Hybrid	AChEI (IC_{50})	BuChE (IC_{50})	Inhibition of Aβ aggregation	Reference
Donezepil-Tacrine  **1**	0.27 ± 0.23 nM	66.3 ± 4.0 nM	$A\beta_{1-40}$ = 46.1 %	[36]
AP2238  **2**	44 ± 0.006 nM	48.9 ± 3.7 μM	$A\beta_{1-42}$ > 50%	[37]
AP2469  **3**	8.60 ± 0.21 μM	124 ± 13 μM	$A\beta_{1-42}$ > 50%	[37]
Donezepil-Ebselen  **4**	97 ± 0.007 nM	-	$A\beta_{1-40}$ = 21.4 %	[38]
Tacrine-Dihydropyridine  **5**	105 ± 15 nM	> 100000 nM	$A\beta_{1-42}$ = 34.9 %	[39]
Tacrine-Ferulic acid  **6**	10.9 nM	17.7 nM	-	[40]
Tacrine-8-hydroxyquinoline	**7** 0.50 ± 0.02 nM **8** 1.0 ± 0.05 nM	6.5 ± 0.3 nM 55 ± 2 nM	19% 27%	[41]

 **7** **8**				
Uperizine A-Tacrine  **9** R= CH_3, R'= NH_2 **10** R= H, R'= NH_2 **11** R= H, R'= COOMe	**9** 16.5 ± 0.3 nM **10** 15.7 ± 0.9 nM **11** 6.4 ± 0.8 nM	20.8 ± 2.6 nM 30.8 ± 2.0 nM 19.5 ± 1.9 nM		[42]
Galantamine-Memantine (Memagal)  **12**	1.16 ± 0.003 nM	-		[43]
Tacrine-Flurbiprofen  **13**	19.3 nM	3.7 nM	$A\beta_{1-40}$ = 31 %	[44]
Lipocrine  **14**	0.25 nM	10.8 nM		[45]
Memoquin  **15**	2.60 ± 0.48 nM	-	$A\beta_{1-40}$ 28.3 μM $A\beta_{1-42}$ 5.93 μM	[46]

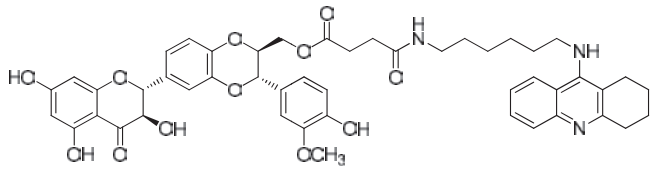


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Figure 2: Structure of tacrine-silibinin codrug.

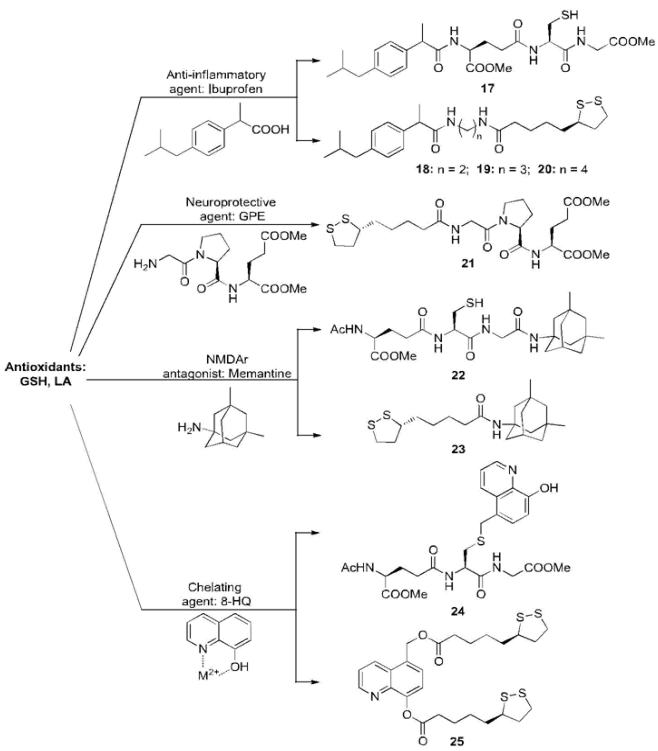

Figure 3: Direct- and spacer-coupled codrug strategy applied to the synthesis of multifunctional ligands for the treatment of AD.

permeation. The pharmacokinetic profiles of codrugs **18-20** revealed a high stability ($t_{1/2}$ > 85 h) in aqueous buffer solutions (pH 1.3 and 7.4) and in human plasma ($t_{1/2}$ > 2 h) (Table 3). Furthermore, IBU-LA codrugs were hydrolyzed more rapidly in brain tissue ($t_{1/2}$ > 13 min) than in human plasma, indicating that these new entities might allow targeted delivery of the parent drugs to damaged neurons [59]. DPPH and DOM experiments evidenced free radical scavenging activities of codrugs **18-20**.

In $A\beta_{1-40}$-infused rat cerebral cortex codrug **18** treatment showed neuroprotective activity at the dose of 10 mg/Kg for 28 days; it resulted able to decrease aggregation of Aβ plaques and expression of $A\beta_{1-40}$ proteins [60], as well as limit NOS activity [61] that is augmented in $A\beta_{1-40}$-perfused rat brains, as reported by Limon *et al* [68]. In the same experimental model, neuroprotective and antiapoptotic roles of codrug **18** on neuroglobin (Ngb) levels have been investigated [62] since Ngb deficiency is associated with age-related neurodegeneration [67]. IBU-LA (**18**) displayed a significant restoration of Ngb levels in Aβ-infused rat cerebral cortex respect to the control through p-Akt and p-CREB recruitment [62].

In 2012 we conjugated LA, via direct-coupled codrug strategy, to another endogenous neuroprotective tripeptide, glycyl-L-prolyl-L-glutamic acid (GPE) that is able to guarantee the release of acetylcholine and dopamine from rat cortex and striatum through stimulation of dopaminergic neurotransmission (Figure 3). In AD experimental models, the neuroprotective effect of GPE seems to be connected to the regulation of intracellular Ca^{2+} signaling and programmed neuronal death [68].

LA-GPE (**21**) showed an adequate pharmacokinetic profile owing to a good chemical stability at pH 1.3 ($t_{1/2}$ = 58 h), 7.4 ($t_{1/2}$ = 217 h), and human plasma ($t_{1/2}$ > 3 h), exhibiting good lipophilicity (logP = 1.51) and water solubility (8.65 mg/mL) (Tables 2-3). Additionally, LA-GPE has been classified as a high CNS permeable compound (P_e > 4 x 10^{-6} cm s^{-1}) using Parallel Artificial Membrane Permeability Assay (PAMPA-BBB), a non-cell based assay to predict the ability of the codrug to diffuse through BBB (Table 2). At the concentration of 100 μM LA-GPE displayed neuroprotective activity against H_2O_2 in SH-SY5Y human neuroblastoma cells through a mechanism not yet defined. Overall, these findings suggest that IBU-GSH, IBU-LA, and LA-GPE may protect against oxidative stress generated by ROS and reduce AD inflammatory state.

Glutathione-Memantine and Lipoic Acid-Memantine Codrugs Against Oxidative Stress and NMDA Excitotoxicity in AD

Another approach for the treatment of AD is to block glutamatergic neurotransmission [69] since an overactivation of NMDA receptors (NMDAr), followed by high intracellular concentrations of Ca^{2+}, is responsible for excitotoxicity. Therefore, NMDAr antagonists can be useful for the cure of pathologies characterized by an overstimulation of NMDAr [70]. As NMDAr antagonist, Memantine (MEM) was approved in 2004 by FDA for the management of late-stage cases of AD; its mechanism of action is based on the modulation of Ca^{2+} influx leaving the channel relatively open for neurotransmission at low stimulation rates [71]. These considerations gave the rationale for developing a new series of codrugs that completed the pharmacological activities of MEM and antioxidant molecules.

Therefore, in 2013 we combined the antiglutamatergic properties of MEM to the radical scavenging activities of antioxidant agents, such as GSH and LA, synthesizing two novel anti-AD codrugs named GSH-MEM (**22**) and LA-MEM (**23**) (Figure 3) [69]. Codrugs **22-23** were obtained by joining GSH or LA, respectively, to MEM via an amide bond using the direct-coupled codrug strategy. GSH-MEM possesses a good water solubility (0.43 mg/mL), whereas LA-MEM seems to be less water soluble (0.02 mg/mL), in agreement with the higher lipophilicity of LA (Table 2). The water solubility and LogP suggested a good absorption profile of codrug **22** after oral administration (Table 2). GSH-MEM showed a good stability toward gastrointestinal hydrolysis ($t_{1/2}$ = 55 h) compared to LA-MEM ($t_{1/2}$ > 6 h) (Table 3). On the contrary, in rat and human plasma GSH-MEM underwent rapid bioconversion into its constituents ($t_{1/2}$ > 1 h in human plasma and immediate hydrolysis in rat plasma), while LA-MEM displayed a good enzymatic stability in both enzymatic environments ($t_{1/2}$ > 5 h in human plasma and > 1 h in rat plasma). Codrug **23** was classified as greatly BBB-permeable (P_e = 3.47 x 10^{-6} cm s^{-1}) while codrug **22** as weakly BBB-permeable (P_e = 1.80 x 10^{-6} cm

Table 2: Physico-chemical properties of codrugs **17-25**.

Codrug	Water Solubility[a]	Lipophilicity			PAMPA-GI P_e (10^{-6} cm s^{-1})[b]			PAMPA-BBB P_e (10^{-6} cm s^{-1})[b]	
	(mg/mL)	LogP[a]	*c*LogP	$LogK_0$	pH 5.0[c]	pH 6.5[c]	pH 7.4[c]	pH 7.4[c]	Classification[d]
17	0.0103 ± 0.9 x 10^{-3}	2.70 ± 0.20	3.02 ± 0.72	3.300 ± 0.140	n.a.	n.a.	n.a.	n.a.	n.a.
18	0.02 ± 0.9 x 10^{-3}	n.a.	4.43 ± 0.53	2.239 ± 0.090	n.a.	n.a.	n.a.	n.a.	n.a.
19	0.02 ± 0.2 x 10^{-3}	n.a.	4.95 ± 0.51	2.387 ± 0.095	n.a.	n.a.	n.a.	n.a.	n.a.
20	0.01 ± 0.4 x 10^{-3}	n.a.	5.71 ± 0.50	2.719 ± 0.057	n.a.	n.a.	n.a.	n.a.	n.a.
21	8.65 ± 0.35	1.51 ± 0.02	1.43 ± 0.01	n.a.	n.a.	6.05	12.63	6.45	CNS +
22	0.43 ± 0.02	2.55 ± 0.01	1.67 ± 0.74	0.610 ± 0.030	n.a.	n.a.	n.a.	1.8	CNS -
23	0.02 ± 0.004	4.20 ± 0.06	5.23 ± 0.46	2.660 ± 0.090	0.51	2.57	10	3.47	CNS +/-
24	12 ± 0.42	n.a.	n.a.	n.a.	7.5	12.9	16	2.8	CNS +/-
25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[a] Values are the means of ± three experiments
[b] Artificial membrane permeability coefficient
[c] pH of both donor and acceptor compartments
[d] 'CNS + ' (high BBB permeation predicted), P_e (10^{-6} cm s^{-1}) > 4.0; 'CNS+ /- ' (BBB uncertain permeation), *Pe* (10^{-6} cm s^{-1}) from 4.0 to 2.0 'CNS – ' (low BBB permeation predicted), P_e (10^{-6} cm s^{-1}) < 2.0; The calculated *c*LogP was determined using ACD LogP software package, version 4.55 (Advanced Chemistry Development Inc., Toronto, Canada); n.a.: Not Available.

Table 3: Kinetic data for chemical and enzymatic hydrolysis of codrugs **17-25** at 37 °C[a].

Codurg	Chemical hydrolysis			Human plasma			Rat Plasma	
	$t_{½}$ (h) pH 1.3	k_{obs} (h^{-1}) pH 1.3	$t_{½}$ (h) pH 7.4	k_{obs} (h^{-1}) pH 7.4	t½ (h)	kobs (h-1)	t½ (h)	kobs (h-1)
17	>16	n.a.	>16	n.a.	>0.75	0.91±0.02	n.a.	n.a.
18	>85	n.a.	>85	n.a.	3.005±0.135	0.23±0.01	1.137±0.023	0.66±0.02
19	>85	n.a.	>85	n.a.	2.020±0.057	0.35±0.01	0.842±0.025	0.84±0.02
20	>85	n.a.	>85	n.a.	1.922±0.070	0.36±0.01	0.718±0.0018	0.97±0.02
21	58.75±1.76	0.0118±0.0002	217.17±2.35	0.0032±0.0001	3.14±0.08	0.22±0.03	0.085±0.004	8.15±0.46
22	55.0±2.4	0.013±0.001	120±1	0.005±0.001	1.57±0.08	0.44±0.02	immediate	n.a.
23	6.32±0.09	0.29±0.01	30.2±1.1	0.023±0.010	5.72±0.23	0.12±0.02	0.99±0.04	0.70±0.02
24	>7	n.a.	15.37±0.32	0.045±0.001	8.50±0.39	0.081±0.001	n.a.	n.a.
25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[a] Values are the means of ± three experiments; n.a.: not available.

s^{-1}), suggesting a good penetration through the BBB and a targeted delivery of LA directly to sites damaged by oxidative stress (Table 2). The antioxidant profiles of **22-23** were confirmed by testing them on GL15 cellular population, an astroglial-like cell line used as an *in vitro* model of astrocytes. At the concentration of 10 μM, both codrugs were able to contrast the intracellular ROS produced by H_2O_2 in GL15 cells similarly to GSH and LA. LA-MEM significantly inhibits $A\beta_{1-42}$ aggregation interfering with the Aβ aggregation process, as evidenced by a ThT fluorimetric *in vitro* assay [73]. These favorable properties make LA-MEM a promising candidate to be screened *in vivo* for its anti-AD activities.

Glutathione-8-Hydroxyquinoline and Bis-Lipoyl-8-Hydroxyquinoline Codrugs Against Oxidative Stress and Metal Dyshomeostasis in AD

High levels of metal ions [Cu(II), Zn(II), and Fe(III)] have been found in Aβ plaques of AD brains. Particularly, Cu(II) and Zn(II), bind to Aβ peptides facilitating Aβ aggregation. Moreover, Cu(II) and Fe(III), either unbound and bound to Aβ peptides, promote overproduction of ROS, well-known for their toxicity [72]. Many reports support the hypothesis that the modulation of biometals in the brain represents a promising therapeutic strategy for the treatment of AD [73-75]. Recently, novel inhibitors able to complex metals were developed with the aim of inhibiting Aβ aggregation [76-78].

Lately, to search chemical tools skilled in reducing oxidative stress and modulating metal dyshomeostasis in AD patients, we designed novel codrugs potentially able to interfere with metal-Aβ and metal-ROS interactions. We focused on molecular combinations obtained by joining GSH and/or LA, as antioxidant molecules (Figure 3), with 8-hydroxyquinoline (8-HQ) that – chelating Cu(II), Fe(III), and Zn(II) – prevents the precipitation of Aβ plaques [79-81].

GS(HQ)H (**24**) is a water soluble (12 mg/mL) codrug, stable in human plasma with a $t_{1/2}$ > 8 h and BBB-permeable (P_e = 2.8 x 10^{-6} cm s^{-1}) (Tables 2-3) [82]. In *in vitro* experiments on SH-SY5Y human neuroblastoma cells – differentiated with Retinoic Acid (RA) to obtain a cholinergic phenotype – codrug **24**, at the concentration of 1 μM, showed a marked prevention of cellular death against H_2O_2-induced oxidative stress. Moreover, the experimental K_D values demonstrated that **24** could be able to remove Cu(II) and Zn(II) from the Aβ peptide, leaving unmodified physiological pools of Cu(II) and Zn(II) *in vivo*. The K_D value obtained for the Cu(II)/ligand system is 280 pmol/L, whereas the one calculated for Zn(II)/ligand system

is 0.57 μmol/L, within the range suggested by Faller and Hureau [83]. The synergic effect of GSH and HQ resulted evident when the two molecules were combined respect to the single compounds. By globally evaluating the activity profile, GS(HQ)H could be one of the earliest chelanting codrugs to test on Aβ-infused rats.

In parallel, codrug LA-HQ-LA (**25**), obtained combining the antioxidant and neuroprotective properties of LA and chelating activities of 8-HQ, was synthesized by linking via two ester bonds the 5-hydroxymethyl-8-hydroxyquinoline to LA (Figure 3) [84]. Codrug **25**, due to its elevated lipophilicity, is able to cross the BBB and possessing two ester bonds of different strength, might slowly and continuously release LA and HQ directly inside the brain. Our outcomes showed that LA-HQ-LA resulted in significant neuroprotective and antioxidant effects against H_2O_2-induced neurotoxicity in human neuroblastoma SH-SY5Y cells differentiated with RA. The metal chelating properties and the pharmacokinetic profile of codrug **25** are currently under study in our research laboratory.

Conclusion

The complexity of AD is suggested to arise from multiple pathological factors, such as oxidative stress, free radicals, metal dyshomeostasis, and neuroinflammation; all these factors contribute in a different manner to the onset of AD. To simultaneously contrast these features, in the last years we have rationally designed novel codrugs by joining antioxidant portions to molecules endowed with antinflammatory, antiglutamatergic, or metal chelating activities into a single framework. These codrugs could diminish the oxidative damage caused by mitochondrial free radicals and delay the degenerative process related to excessive deposition of Aβ, metal accumulation, or glutamate excitotoxicity. These properties highlight that our multifunctional ligands could be interesting in the search for new disease-modifying agents for AD. In our upcoming research, we intend to widen this work by testing *in vivo* all the synthesized codrugs and preparing nanotechnologic formulations to improve their pharmacokinetic profiles, thus rendering them attractive tools for future pharmacological applications.

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