Lipoic Acid, a Lead Structure for Multi-Target-Directed Drugs for Neurodegeneration

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Abstract. Nowadays, drug discovery based on a single-target-directed strategy seems inappropriate for the treatment of complex diseases that have multiple pathogenic factors. Recent research into new drugs, which are able to hit different targets, highlights the idea that a single molecule could be sufficient to treat multi-factorial diseases. In this review, examples of multi-target-directed compounds derived from lipoic acid are examined.

Key Words: Neurodegenerative diseases, Lipoic acid, Antioxidants, Multi-target-directed drugs, Reactive oxygen species.

1. WHICH STRATEGY FOR DRUG DISCOVERY?

Drug discovery has been and still is a formidable challenge to the scientific community. Despite the efforts of generations of medicinal chemists and biologists, effective drugs to safely treat certain diseases remain a dream. Drug development has been historically based on a single-target-directed compound strategy. Nowadays, this paradigm appears inadequate for those diseases that have multiple pathogenic factors, such as, among others, neurodegenerative diseases, diabetes, cardiovascular disease, and cancer [1,2]. To overcome the problems arising with drugs that hit a single target, combinations of different drugs or drug cocktails are now used to treat particular diseases, such as, for example, neoplastic disorders, where they achieve maximum efficacy by attacking several targets simultaneously, exploiting synergy and minimizing individual toxicity. However, a new strategy, based on the assumption that a single compound may be able to hit multiple targets (multiple-target-directed compound), is emerging in drug discovery [3-6]. The problem is: how can multiple-target-directed compounds be developed? A potential answer may be found in a particular chemical structure (privileged structure) able to alter a general biological process, which may represent a pathogenic factor common to different diseases. The insertion of appropriate pharmacophores onto this structure may enable the new chemical entity to hit selected biological targets [7]. This may be considered a follow-up of the strategy in which two distinct pharmacophores of different drugs are combined in the same structure leading to hybrid molecules. In principle, each pharmacophore of these new molecular entities would retain the ability to interact with its specific site(s) on the target(s) and, consequently, produce multiple specific pharmacological responses that, taken together, would be useful for the treatment of multi-factorial diseases.

2. SELECTION OF A PRIVILEGED STRUCTURE

The privileged structure concept has emerged as a useful approach to the design and discovery of new drugs [8,9]. A privileged structure is a molecular scaffold able to provide potent and selective ligands for a range of different biological targets through the modification or insertion of particular functional groups. In addition, privileged structures should possess good drug-like properties, which in turn should lead to more drug-like compounds.

The selection of a suitable privileged structure for multifactorial diseases should begin by considering which are the pathogenic factors leading to the disease. Since oxidative stress appears to play a pathogenic role in all the neurodegenerative conditions [10] exemplified by Alzheimer's disease (AD), Lewy body diseases such as Parkinson's disease (PD), amyotrophic lateral sclerosis, and Huntington's disease, it follows that an ideal privileged structure for these diseases might well be one with antioxidant properties. There is growing interest in antioxidants as a protective strategy against the pathologies associated with oxidative stress, which can be broadly defined as an imbalance between oxidant production and cells' antioxidant capacity to prevent oxidative injuries. This phenomenon is imputable to high levels of reactive oxygen (ROS) and reactive nitric oxide species.

The next step in the design strategy for the development of potential new drugs would be the insertion of appropriate functional groups onto the selected privileged structure in order to achieve selectivity for a given disease; in other words, to address the drug towards given targets.

3. LIPOIC ACID AND OXIDATIVE STRESS

Lipoic acid is known as a universal antioxidant [11-14]. Its antioxidant activity is attributed to the capacity to scavenge a number of free radicals in both membrane and aqueous domains, by chelating transition metals in biological systems, thus preventing membrane lipid peroxidation and protein damage through the redox regeneration of endogenous antioxidants such as vitamin E (α-tocopherol), vitamin C (ascorbic acid) and notably gluthathione (Fig. (**1**)), thus maintaining an intracellular antioxidant balance [15]. They have been extensively studied as useful neuroprotective agents [16-18]. However, in contrast to lipoic acid, their use as therapeutic agents is limited because of their marginal ability to cross the blood-brain barrier or because, in the case

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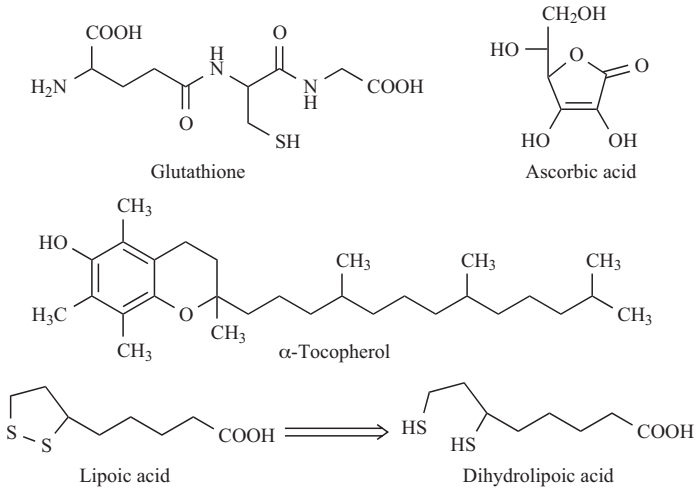


Fig. (1). Chemical structure of the natural antioxidants glutathione, ascorbic acid (vitamin C), and α-tocopherol (vitamin E) and of lipoic acid and its reduced metabolite dihydrolipoic acid.

of ascorbic acid or α-tocopherol, they do not affect iron accumulation and, consequently, have only limited efficacy in lowering the oxidative stress in the aging brain [19,20]. Lipoic acid is readily absorbed by diet, transported, taken up by cells, and reduced to dihydrolipoic acid (DLA) in various tissues, including brain. It has been demonstrated that dihydrolipoic is an even more potent antioxidant than lipoic acid.

A variety of investigations performed in recent years have highlighted the importance of the protective effects exerted by lipoic acid in neurodegenerative diseases. For example:

- It protects neurons against cytotoxicity induced by amyloid-β (Aβ) [21] and stabilizes cognitive functions in patients with AD [22].
- It increases glutathione levels, which may prove helpful in preventing the antioxidant depletion in PD [23].
- Together with DLA it dose-dependently inhibits the formation and extension of Aβ fibrils from Aβ [24].
- It ameliorates the neurotoxicity which arises from metabolic compromise induced by inhibitors such as 3-nitropropionic acid, rotenone, and 1-methyl-4-phenylpyridinium in animal models. These models are thought to simulate the processes that may lead to diseases such as Huntington's and Parkinson's diseases [25,26].
- It combats cognitive dysfunction and neurodegeneration induced by D-galactose exposure, and also reduces peripheral oxidative damage by decreasing malondialdehyde and increasing total antioxidative capabilities and total superoxide dismutase, without affecting glutathione peroxidase activity [27].

4. LIPOIC ACID AND MULTI-TARGET-DIRECTED COMPOUNDS

Clearly, lipoic acid has multiple biological properties and could represent the basis for designing new drugs for the investigation and, hopefully, treatment of complex diseases that have multiple pathogenic factors such as, among others, AD and PD. Thus, to design a drug around the base of lipoic acid, it becomes imperative to select the pharmacophoric groups to be appended onto the privileged structure in order to hit the different targets that are known to play important roles in the pathogenesis of the given disease.

AD, the most common cause of dementia, is a complex neurological affliction, which is clinically characterized by loss of memory and progressive deficits in different cognitive domains. The consistent neuropathological hallmark of the disorder, generally noted on postmortem brain examination, is a massive deposit of aggregated protein breakdown products, Aβ plaques and neurofibrillary tangles. Although the primary cause of AD is still uncertain, Aβ aggregates are thought to be mainly responsible for the devastating clinical effects of the disease [28]. In recent years, significant research has also been devoted to the role of free radical formation, oxidative cell damage, and inflammation in the pathogenesis of AD, providing new promising targets and validated animal models [29]. To date, however, the enhancement of the central cholinergic function is the only clinically effective approach [30,31]. The intensive research into drugs able to improve the cholinergic transmission in AD has produced so far four approved acetylcholinesterase (AChE) inhibitors, that is, tacrine [32], donepezil [33], rivastigmine [34], and galantamine [35]. However, these drugs have been approved for the symptomatic treatment of AD only as they do not address the etiology of the disease for which they are used.

Very recently, it has been suggested that, for the treatment of AD, it would be preferable to have pharmacological tools that are able to act as far upstream as possible in the neurodegenerative cascade and able to hit different selected targets. To this end, the structure of lipoic acid was combined with a pharmacophore that had well-established biological properties, namely, the ability to inhibit AChE activity [36]. It was also argued that the cyclic moiety of lipoic acid could be able to interact with the peripheral anionic site

of AChE, which is associated with the neurotoxic cascade of AD through AChE-induced Aβ aggregation. Thus, this strategy allowed the antioxidant properties of lipoic acid to be combined into the same molecule with the abilities of classical AChE inhibitors (such as tacrine) to improve cholinergic transmission and inhibit Aβ aggregation. This led to the creation of a new class of compounds, whose prototype is lipocrine (Fig. (**2**)). Lipocrine emerged as a candidate for drug development, displaying multiple biological properties, namely, inhibition of AChE activity, inhibition of AChE-induced Aβ aggregation, and ability to protect cells against ROS (Table **1**) [36].

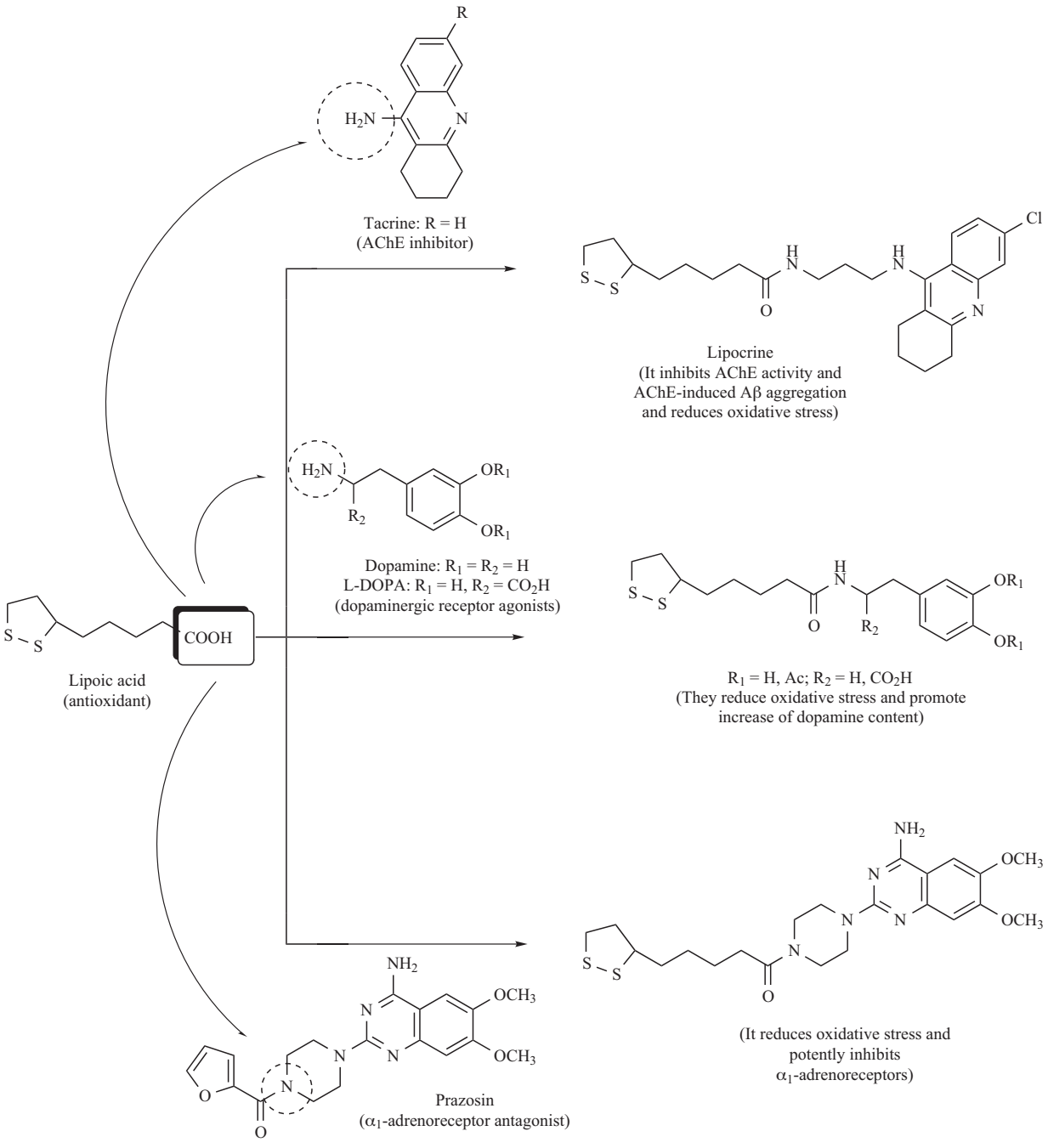
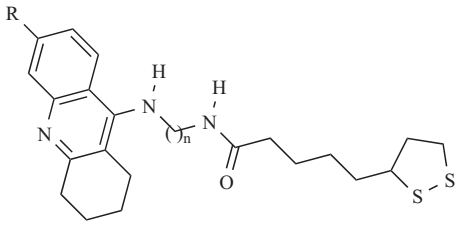


Fig. (2). Chemical structure of selected examples of multi-target-directed compounds. The design strategy leading to these compounds is outlined by linking the structure of lipoic acid to that of an appropriate pharmacophore. Lipoic acid represents the privileged structure on which the selected pharmacophore is inserted to achieve selectivity for given targets.

Table 1. Inhibition of Human Recombinant AChE Activity, AChE-Induced Aβ Aggregation, and Intracellular ROS by Lipocrine (7) and Homologues in Comparison with Prototypes Tacrine Analog 8 and Lipoic acid (LA)[a]



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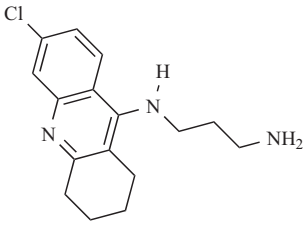

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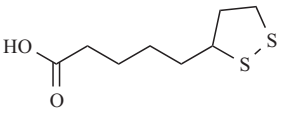


LA

Compound	n	R	IC_{50} (nM) AChE	IC_{50} (μM) Aβ aggregation	% increase of intracellular ROS (μM)						
					0	0.1	0.5	1	5	10	50
1	2	H	97.0								
2	3	H	6.96								
3	4	H	35.2								
4	5	H	38.4								
5	6	H	30.1								
6	7	H	32.7								
7	3	Cl	0.253	45.0	86	89	95	77	62[d]	51[e]	30[e]
8			21.5	> 100[b]	86	99	100	97	82	tox[f]	tox[f]
LA			>1000000	na[c]	86	91	90	83	79	74	58[d]
Propidium			32300	12.6							

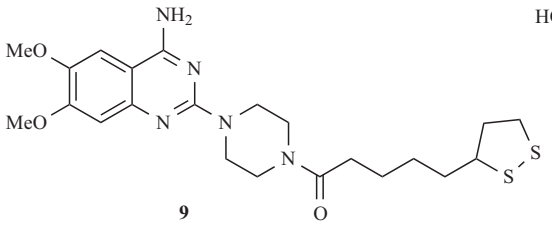
[a]Data taken from [36,37]. [b]At this concentration 25% inhibition of Aβ aggregation was observed. [c]na, not active at 100 μM. [d]$p < 0.01$. [e]$p < 0.001$. [f]tox, citotoxicity.

The results assembled in Table **1** clearly show that lipocrine is a potent inhibitor of AChE activity with a subnanomolar IC_{50} value. Furthermore, lipocrine is an effective inhibitor of AChE-induced Aβ aggregation with an IC_{50} value of 45.0 μM that is only 3-fold higher than that displayed by propidium (12.6 μM), which is one of the most potent inhibitor of AChE-induced Aβ aggregation so far available [37]. Interestingly, tacrine and lipoic acid, i.e., the pharmacophoric moieties combined in lipocrine, were not able to inhibit at 100 μM concentration the Aβ aggregation induced by AChE while tacrine analog **8** gave at the same concentration an inhibition of only 25%. In this context, it is relevant that also an association of 100 μM lipoic acid and 100 μM **8** produced only a weak inhibition (30%) of AChE-induced Aβ aggregation, suggesting that marked Aβ aggregation inhibition may be achieved only when the two prototypes are combined into the same structure, as in lipocrine, achieving the ability to inhibit AChE peripheral anionic site, which is connected with Aβ aggregation process [36]. Finally, it was also demonstrated that both lipocrine and lipoic acid, unlike tacrine analog **8**, did not affect neuronal viability and were able to protect neuronal cells against ROS formation evoked by oxidative stress, with lipocrine being the most active against ROS formation (Table **1**) [36].

PD, like AD, is another neurodegerative disease in which an oxidative stress hypothesis for disease pathogenesis is well-documented [23]. PD is associated primarily with loss of dopaminergic neurons in the nigrostriatal system. It appears that dopaminergic neurons may be damaged in PD by highly reactive hydroxyl radicals, perhaps arising from the Haber-Weiss or Fenton reactions. The oxidative stress hypothesis is further supported by the increased production of the superoxide anion radical in the mitochondria and increased superoxide dismutase activity in the substantia nigra from Parkinson's individuals. There are a number of sites in the chain of oxidative stress reactions where lipoic acid or its reduced metabolite, dihydrolipoic acid, can be effective. In particular, lipoic acid can remove superoxide and hydroxyl radicals interrupting the chain of degenerative reactions at key points [23].

Recently, it was suggested that joining an antioxidant molecule to a pharmacophore that is able to target a specific population of dying cells would be useful in the search for effective treatments of PD. Thus, the structure of dopamine or L-DOPA was linked to that of the antioxidant lipoic acid, leading to new molecules that should be useful dopaminergic agents devoid of the pro-oxidant effects associated with the presence of the catechol moiety (Fig. (**2**)) [38]. These com-

Table 2. Affinity Constants at α_1-Adrenoreceptor Subtypes (pA_2 Values) on Isolated Prostatic Vas Deferens (α_{1A}), Spleen (α_{1B}), and Thoracic Aorta (α_{1D}) and at Human Recombinant α_1-Adrenoreceptor Subtypes (pK_i Values, CHO Cells) and Inhibition of ROS Formation Evoked by Exposure of Cells to *t*-BuOOH of Derivative 9 in Comparison with Prototypes Lipoic Acid (LA) and Prazosin (PR)[a]



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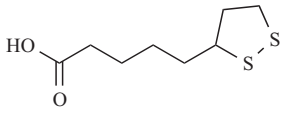
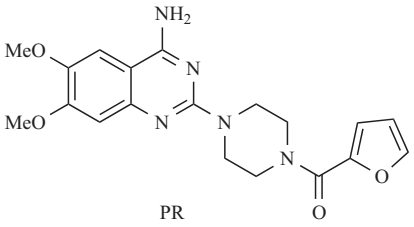


LA



PR

No.	pA_2			pK_i			Antioxidant activity
	α_{1A}	α_{1B}	α_{1D}	α_{1a}	α_{1b}	α_{1d}	IC_{50} (μM)
9	7.19	7.39	7.92	8.85	8.90	9.05	8353
LA	< 5	< 5	< 5	< 5	< 5	< 5	54.86
PR	8.98	9.01	9.01	9.23	9.39	9.65	Not active at 100 μM

[a]Data from [45].

pounds displayed an antioxidant effect and were shown to release, after oral administration in rats, L-DOPA. This may be of relevance for possible therapeutic application in pathological events involving free radical damage and decreasing dopamine concentration in the brain [38].

Lipoic acid may represent a useful starting point for the development of drugs targeted at complex diseases that are not characterized by neurodegenerative conditions. In targeting hypertension, to name one example, lipoic acid's antioxidant properties may have antihypertensive effects [39]. Blood pressure reduction can be achieved by antagonizing α_1-adrenoreceptors [40] with the advantage of a favorable effect on plasma lipoproteins and a low incidence of sexual dysfunction [41]. Furthermore, the activation of these receptors may be responsible for ischemia-induced cardiac arrhythmia [42,43]. Therefore, α_1-adrenoreceptor antagonists could be also useful against this specific pathology. Prazosin, the prototype of quinazoline-bearing compounds, is widely used as a pharmacological tool for the characterization of α_1-adrenoreceptor subtypes and as an effective drug in the management of hypertension [44]. With the aim of widening the biological profile of prazosin, its structure was combined with that of lipoic acid to achieve derivatives endowed with both α_1-adrenoreceptor antagonist and antioxidant properties (Fig. (**2**)). As expected, the obtained compound displayed a potent α_1-adrenoreceptor antagonism and the capacity to inhibit oxidative stress (Table **2**) [45]. Clearly, compounds with these properties may be of relevance to the management of cardiovascular disease.

CONCLUSION

The pharmaceutical community has made significant strides in understanding the mechanisms underlying the pathogenesis of neurodegenerative diseases, and in developing different classes of ligands directed at them. However, all the drugs currently available, including those in experimental trials, even with different mechanisms of action, are monofunctional, targeting only one of the many processes involved in neurodegeneration. It is very likely that syndromes such as AD require multiple-action drugs to address the various pathological aspects of the disease. Although the use of combinations showed early promise and has been widely exploited by the industry, this research field has yielded few definitive successes. The development of multi-target-directed drugs appears to offer better therapeutic potential. Clearly, therapy with a single drug that has multiple biological properties has inherent advantages over combinations of drugs. In fact, multi-target-directed drugs will obviate the challenge of administering multiple single-drug entities, which may have different bioavailability, pharmacokinetics, and metabolism. Furthermore, multi-target-directed drugs will also simplify the therapeutic regimen for AD patients with compliance problems [46,47]. Lipoic acid has been shown to be a suitable privileged structure for the design and development of multi-target-directed compounds that may be useful to treat those multi-factorial diseases in which oxidative stress plays a pathogenic role.

ACKNOWLEDGEMENTS

This work was supported by MIUR, Rome (Grant n. Firb RBNE03FH5Y).

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Received: June 01, 2006 **Revised: June 09, 2006** **Accepted: June 10, 2006**