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Multitarget ligands and theranostics: sharpening the medicinal chemistry sword against prion diseases

Prion diseases (PrDs) are fatal neurodegenerative disorders, for which no effective therapeutic and diagnostic tools exist. The main pathogenic event has been identified as the misfolding of a disease-associated prion protein. Nevertheless, pathogenesis seems to involve an intricate array of concomitant processes. Thus, it may be unlikely that drugs acting on single targets can effectively control PrDs. In addition, diagnosis occurs late in the disease process, by which point it is difficult to determine a successful therapeutic intervention. In this context, multitarget ligands (MTLs) and theranostic ligands (TLs) emerge for their potential to effectively cure and diagnose PrDs. In this review, we discuss the medicinal chemistry challenges of identifying novel MTLs and TLs against PrDs, and envision their impact on prion drug discovery.

Keywords: bivalent ligands • Creutzfeldt–Jakob disease • prion drug discovery • protein misfolding diseases • rare diseases

Prion diseases (PrDs) or transmissible spongiform encephalopathies (TSEs), are a family of neurological disorders that include Creutzfeldt–Jakob disease (CJD) and Gerstmann–Sträussler–Scheinker disease (GSS) in humans, chronic wasting disease in deer and scrapie in sheep [1,2]. While CJD is a rare human disease affecting approximately one in a million people worldwide, it invariably proves fatal [2].

Scientific and public interest in PrDs has increased dramatically in recent years, transforming them from rare, enigmatic infectious diseases to a sort of emblem of neurodegeneration [3]. Initially, what made them so unique and fascinating to scientists was the growing understanding that the infectious agent responsible for PrDs was different from conventional microorganisms [4]. In past years, many discoveries provided compelling evidence in favor of Prusiner's revolutionary 'prion only hypothesis', namely that a misfolded protein is the main (and perhaps the sole) component of the prion infectious agent [5]. In PrDs the normally expressed protein (PrP^C) is converted into its toxic iso-

form termed PrP^{Sc}, which is rich in β -sheet secondary structures, insoluble and resistant to proteolytic digestion [6,7]. Through autocatalytic replication, PrP^{Sc} accumulates in proteinaceous aggregates, resulting in the diffuse neuronal degeneration and the spongiform brain lesions typical of the disease [8]. Although the exact molecular mechanism of PrP^C to PrP^{Sc} conversion is still not clear, it has been proposed that the misfolded PrP^{Sc} acts as a template that induces the normally folded proteins to change their conformation [5,9].

Additionally, PrDs have been under the spotlight because of the bovine spongiform encephalopathy (BSE) epidemic, which started in the mid-1980s in UK, spreading to other European countries [10]. Transmission of BSE to humans is believed to have caused ≥ 200 cases of variant CJD (vCJD) worldwide (Figure 1).

Recently PrDs have again come to the forefront of biomedical research, largely spearheaded by a fascinating new hypothesis that is garnering growing support. Several lines of evidence suggest that all proteins causing neurodegeneration are prions, in the sense

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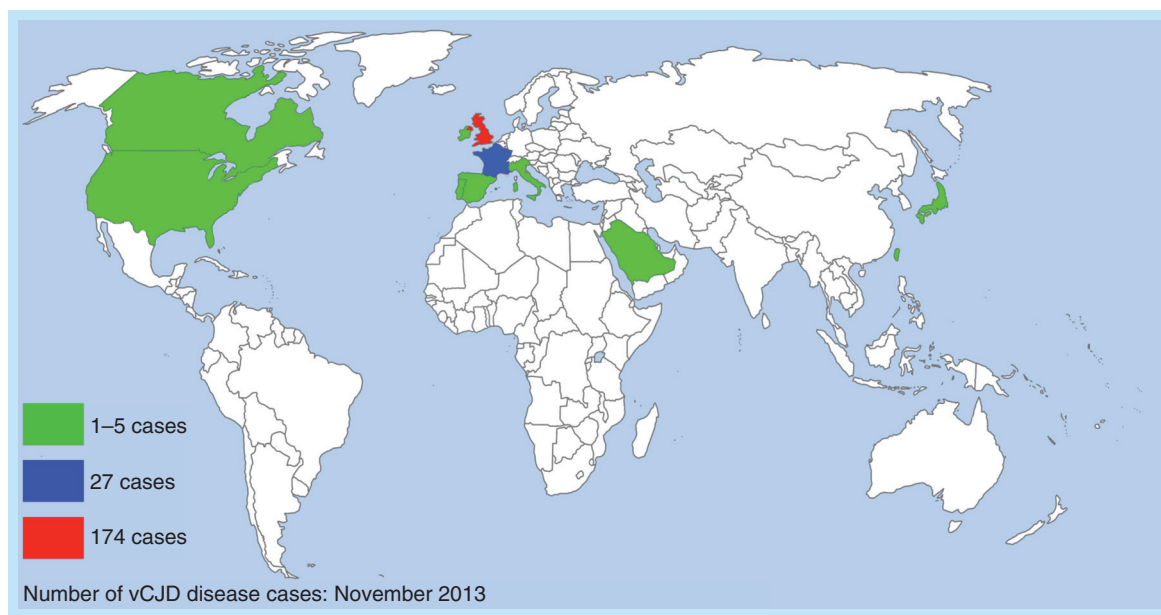


Figure 1. Geographic distribution of variant Creutzfeldt–Jakob disease. vCJD occurs mainly in UK and France. The map was built from cases reported on the vCJD website [11]. Updated to November 2013. vCJD: Variant Creutzfeldt–Jakob disease.

that misfolded proteins, such as β -amyloid ($A\beta$) in Alzheimer's disease (AD) or α -synuclein in Parkinson's disease, could have a prion-like mechanism of propagation and infection [12,13]. Nevertheless, further experimental proofs are needed to confirm such theory, that so far seems to be premature ignoring a great deal of evidence, especially regarding AD etiology [14].

Although these early reports must be treated with due caution, it is not heretical to say that the concepts of aberrant protein folding and templating, first developed for a niche disease, may apply to an array of neurodegenerative diseases representing the major public health challenge of our time [12,15]. In this perspective, drug discovery in the area of PrDs acquires a broader interest than it ever possessed before.

Regrettably, despite the great advances that have occurred so far and the potential impact of future therapies, PrDs, as well the other neurodegenerative diseases, remain incurable and a challenging endeavor for medicinal chemists [3,16]. Paradoxically, in recent decades we have seen an inverse relationship between investments in drug discovery research and successful new drugs. As a result, to date treatments able to definitely arrest the course of disease do not yet exist for any of these disorders [17]. This situation has been attributed to several hurdles that apply not only to PrDs, but to neurodegenerative diseases in general:

- Etiology is not well understood and consequently no therapeutic target has been validated – although drug discovery in this area appears less amenable

to target-based approaches than it seems for other types of therapeutics [18];

- Another reason for the high attrition of drugs in the clinic has been attributed to the poor predictive power of animal models for efficacy in humans [19];
- The failure of many drug candidates has been related to their late administration when the pathology is too advanced [20].

All these inherent difficulties have somehow led medicinal chemists to neglect rational approaches and rather to consider screening approaches as the method of election when looking for new drugs.

In our opinion, in the medicinal chemistry toolbox there is still a multitude of intriguing ideas on how potentially to target PrDs, effectively overcoming the challenges posed by the current shortages. One such option is a multitarget drug discovery approach, which is being successfully pursued in the therapy of other neurodegenerative diseases [21–26]. It involves single chemical entities that interact simultaneously with multiple targets, the so-called multitarget drugs. This polypharmacological approach is in principle more promising because it is more adequate at addressing the multifactorial and progressive pathophysiological processes involved in neurodegeneration [21,27]. Another possibility is the exploitation of theranostics, that is, single agents with concomitant therapeutic and diag-

nostic properties. The general purpose of theranostics is to optimize the efficacy and safety of therapy, as well as to streamline the whole drug-discovery process [28,29]. Along these lines, they seem particularly suitable towards neurodegenerative PrDs [30]. By way of illustration of these principles, in this review, using examples taken from our research, we will propose multitarget ligands (MTLs) and theranostic ligands (TLs) as innovative tools for addressing the pitfalls of current prion drug discovery.

From bivalent to MTLs

As highlighted in the introduction, PrP^{Sc} plays a pivotal role in the onset and propagation of PrDs, even though it is increasingly clear that other mechanisms are strictly interconnected in the underlying neurodegeneration [31]. In the years, several reports have suggested that small molecules that directly target the proteins involved in the fibrillization process represent valid therapeutic tools against prion replication [32]. In this regard, drug discovery approaches that have been widely employed consisted in developing molecules able to interfere with protein–protein interactions (PPIs) between PrP^{Sc} and PrP^C, to avoid prion propagation or to directly prevent fibril formation [33].

Modulation of PPIs is currently regarded as technically undruggable – or at the very least as extremely challenging to target using small molecules: it does not present defined active sites or pockets as in the case of enzymes and receptors, whereas in PPIs large and extended surfaces are involved, and a complex network of weak interactions takes place [34]; high-resolution structural information on amyloid fibrils and aggregates is very scarce, and is currently almost exclusively restricted to those formed by small peptides. In fact, small peptides mimicking the central domain of PrP^C have been extensively used as a model for investigating antiprion effects of small molecules, cyclopeptides and metal complexes [35–40]. The paucity of structural data is mostly due to the inability of crystallography and NMR to address proteins that are by nature insoluble and noncrystalline. Furthermore, characterization of the transient states of such proteins remains challenging because these techniques are not even suitable for studying short-lived protein states [33]. However, the molecular structures of promazine and chlorpromazine bound to a binding pocket formed at the intersection of the structured and the unstructured domains of the mouse prion protein has been recently reported [41].

Based on these considerations, it emerges that the rational design of antiprion compounds still represents a challenge for medicinal chemists. However, considering the oligomeric and repetitive structure of fibrillar aggregates, it was deemed conceivable that a bivalent

molecule, endowed with two identical protein recognition motifs (PRMs) connected by a spacer (Figure 2A), could interact simultaneously with two prion binding surfaces (hot spots), achieving higher potency and enhancing significantly the recognition process [42].

In the light of these considerations, our research group focused on a bivalent strategy to identify novel ligands to contrast TSEs (Figure 2A) [42]. In the first application of these concepts, we looked for a spacer with the ideal features to interact with protein surfaces, that is, a peptidomimetic. The peptidic nature of diketopiperazines (DKPs; Figure 2B) and their occurrence in biologically active natural products has inspired the use of DKP scaffolds as privileged structures for molecular recognition process [43]. Thus, a library of compounds was prepared by condensing 1,4-diacetyl-2,5-piperazinedione with several aromatic aldehydes, giving rise to a new series of DKP-based bivalent ligands [44]. The choice of PRMs included quinolines and pyridines, which are frequently observed in antiprion compounds and are known to have a critical role in PPIs [45]. According to the proposed rationale, compound **1** and **2** (Figure 2B) were found to inhibit prion accumulation in a scrapie-infected cell culture model with an EC₅₀ of 4.1 and 15.8 μM, respectively. Therefore, we confirmed that the DKP core could be a valid scaffold for interfering with PPIs in PrDs. In addition, a planar conformation was fundamental for the activity of these bivalent ligands. Indeed, compounds **1** and **2**, which adopt a planar structure due to an intramolecular hydrogen bonding, resulted effective, whereas in the case of less planar analogues the antiprion activity was lost [44].

Considering the spacer as an important determinant for antiprion activity, we generated a second small library of bivalent ligands by attaching seven amino acids methyl esters to two different 2,5-bis-diaminobenzoquinone (BQ; Figure 2B) central cores [46]. The selection of BQ was motivated by the fact that it is a planar system that should properly interfere with the aromatic residues involved in fibril formation [47]. Furthermore, BQ derivatives had been demonstrated to be able to bind Aβ plaques and affect the native ability of Aβ to self-assemble by disrupting PPIs [48]. On the other hand, it seemed reasonable that aromatic amino acid fragments, such as Trp, Phe and Tyr, could promote the molecular recognition process providing hydrogen-bonds, van der Waals and π–π stacking interactions. As expected, in the cell culture model the phenylalanine derivatives **3** and **4** (Figure 2B), showed the best antiprion activity, displaying EC₅₀ values of 0.9 and 3.6 μM, respectively. In addition, a chemoinformatic analysis confirmed that a BQ central scaffold connected to two aromatic rings is a suitable motif for designing novel antiprion compounds [46].

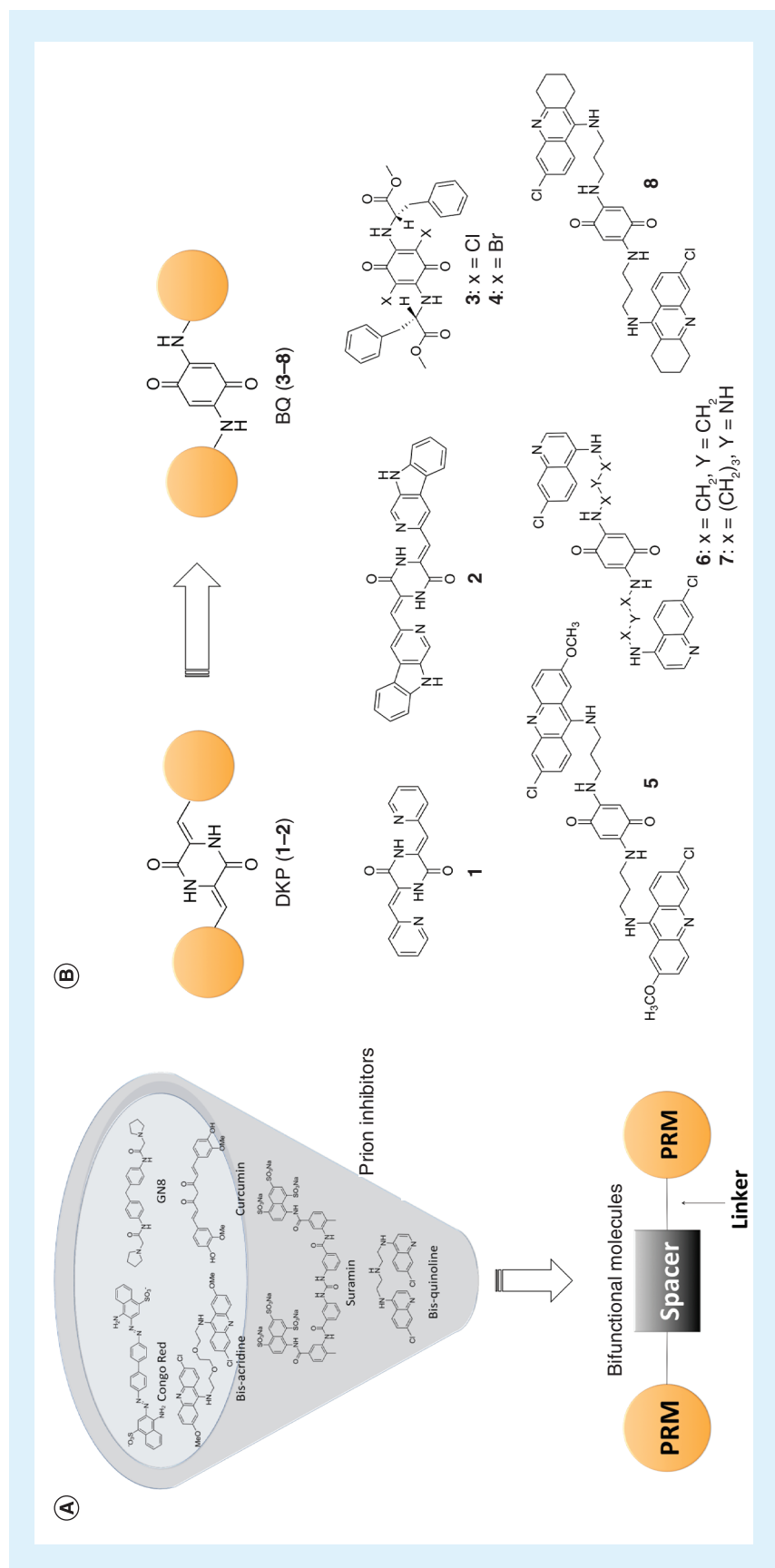


Figure 2. Design strategy leading to bifunctional derivatives. (A) Derivatives able to modulate protein–protein interactions in prion diseases. (B) Chemical structure of DKP (1–2) and BQ-based (3–8) bivalent ligands. BQ: 2,5-bis-diaminobenzoquinone; DKP: Diketopiperazine; PRM: Protein recognition motif.

Considering the BQ nucleus as particularly suitable for perturbing PPIs, we prepared a further series of bivalent ligands [49]. Thus, two active terminal moieties were attached at the positions 2 and 5 of the central BQ core through proper linkers. As PRMs acridine, quinoline and 1,2,3,4-tetrahydroacridine systems were chosen due to the fact that these aromatic heterocycles had been shown to have antiprion potential [45]. On the other hand, three different polyamine chains linkers were selected to explore different lengths and chemical compositions [49]. Compounds **5–8** (Figure 2B) showed remarkably potency against prion replication in cultured cells, with EC₅₀ values ranging from 0.2 to 0.7 μM. The obtained results suggested that a specific length of the linker and the presence of a chlorine atom on the terminal residues are critical for the antiprion activity. Furthermore, to elucidate the mechanism of action of our ligands, the capability of inhibiting prion fibril formation was studied *in vitro* by using an amyloid seeding assay (ASA) [50]. Interestingly, only compounds **6** and **8** delayed fibril formation, and it was proposed that the active molecules probably interact with the recombinant PrP^C and avoid its folding into PrP^{Sc}. Furthermore, based on the well-known antioxidant properties of benzoquinones, their ability to reduce oxidative stress (OS) in PrP^{Sc}-infected cells was investigated. As expected, compound **8** showed a remarkable antioxidant capacity, not different from that of the reference antioxidant Trolox [49]. Thus, compound **8**'s peculiar antiprion mechanism of action results from its bivalent structure, which promotes the interaction with prion recognition domains, whereas the spacer acts simultaneously as a disrupting element against PPIs and an effective antioxidant moiety. These findings led us to speculate that the simultaneous inhibition of prion aggregation and OS would facilitate the creation of effective antiprion agents. In other words, the results of this study paved the way towards the application of the multitarget concepts in the search of new lead candidates against PrDs [49].

The rational or serendipitous discovery of novel chemical entities able to address multiple targets critically involved in a complex pathology is a recent trend of drug discovery. Cancer, depression, schizophrenia and neurodegenerative disorders are all examples of multifactorial diseases for which several MTLs have been developed [21–23,51–53]. A secret weapon against neurodegenerative diseases might be destined to be a MTLs [23,54]. Indeed, Ladostigil (TV-3326), a MTL developed by Youdim and coworkers, is currently in Phase II clinical trials for AD [55].

The most common rational strategy to generate MTL hit compounds is the framework combination [56,57], which starts with the selection of two structures or substructures interfering with two distinct pathological tar-

gets (PTs) or pathological events (PEs) responsible of a given pathology. The two compounds might bind with high selectivity to two different protein targets (PT1 and PT2, see Figure 3A) or modulate two PEs (PE1 and PE2, see Figure 3A). PEs are restored by an action associated to the intrinsic chemical structure of the framework, that is, OS or unbalance of metal ions (see Figure 3D). A combination between a framework binding a PT and another modifying a PE (an antioxidant or a metal chelating group) is also feasible (Figure 3B & C). The final goal is to combine the starting fragments, into the new single molecule by amalgamating them as much as possible to limit the molecular weight increase [58]. In fact, MTLs arising from framework combination can be viewed as linked, fused, or merged depending upon the degree to which the frameworks have been integrated [56,57]. In linked MTLs (Figure 3A–D), the molecular fragments are joined by a chemical linker (either cleavable or metabolically stable). Cleavable linkers contain a group that can be hydrolyzed by plasma enzymes to release the two individual frameworks that then act independently [59]. Metabolic stable linkers have also been reported [60,61]. Fused MTLs are referred to compounds where the two frameworks are connected without a discernable linker, meanwhile merged MTLs indicates an overlap of a common chemical moiety present in both starting fragments. A more extensive discussion on these approaches is out of the scope of this review and might be found in [58,62–65].

We turned our attention to the linked strategy. Thus, each pharmacophore of the new MTLs would preserve the capacity of modulating PTs or PEs, and in turn generate multiple specific pharmacological responses that would enable the successful treatment of PrDs.

The selection of the PTs or PEs is one of the most critical steps in designing novel MTLs. To this respect, a better understanding of which targets are therapeutically relevant is fundamental for the development of more efficacious drugs, either single or multitarget ones. Despite not being fully elucidated, the mechanism underlying PrDs pathogenesis has been associated with a complex array of processes operating simultaneously and synergistically [31]. These include: PrP^C→PrP^{Sc} conversion [5,66]; PrP^{Sc} aggregation [8,67]; OS, accompanied by lipid and protein oxidation [68–72] and reduced levels of potent free-radical scavenger, such as polyunsaturated fatty acids, α-tocopherol, and glutathione [73]; unbalance of metal ions [74]; and brain inflammation with activation of astrocytes and microglia [75].

Preventing or reducing the rate of PrP^C→PrP^{Sc} conversion *in vitro* and *in vivo* is currently the major strategy against PrDs [32,76–84]. This has been achieved by ligands binding the structured part of PrP^C and able to form a stable protein–ligand complex that blocks the confor-

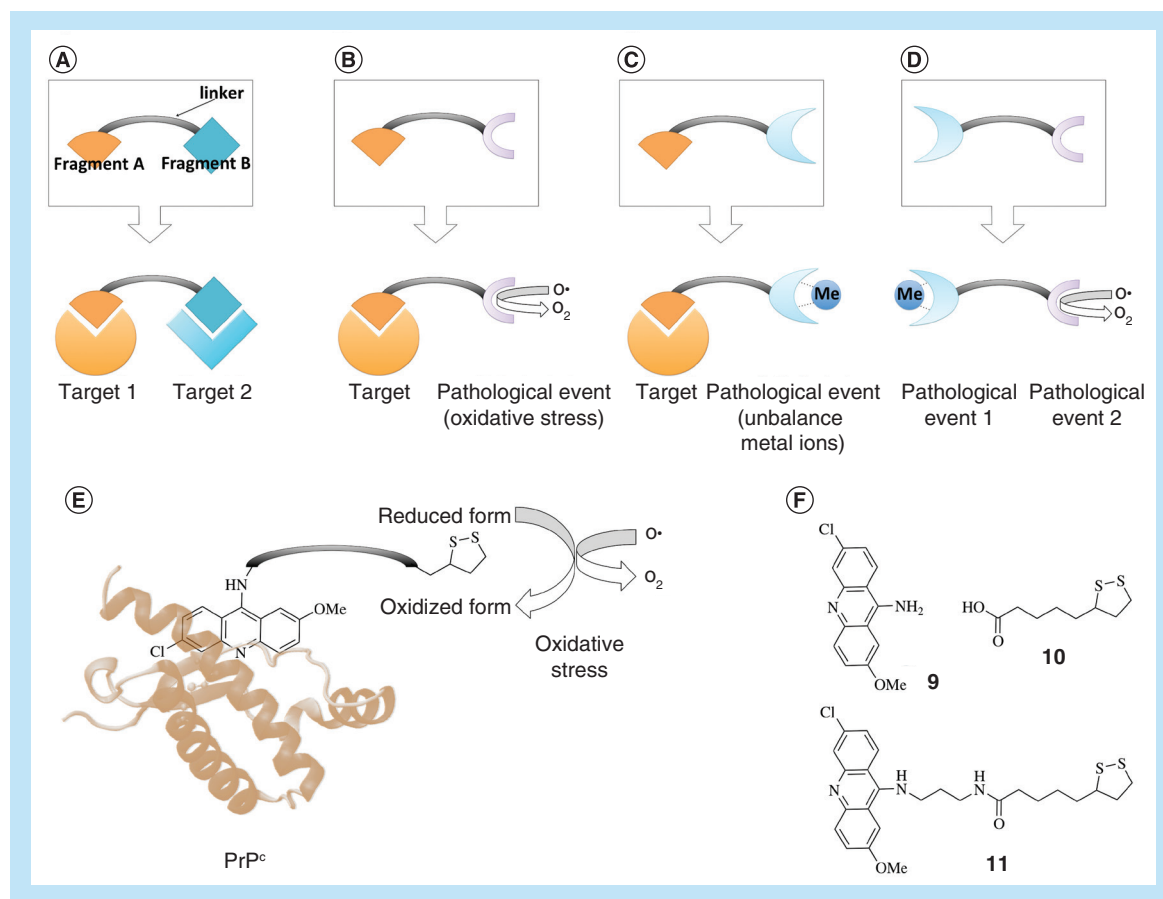


Figure 3. Rational strategy to generate multitarget ligand hit compounds against prion diseases.

(A–D) Multitarget ligands designed by framework combinations (linked strategy). (E) Mechanisms of action of a multitarget. (F) Chemical structure of a multitarget ligand (11) and its starting fragments (9 & 10).

mational change into PrP^{Sc} (Figure 3E) [79–81,83–88]. The protein–ligand complex might lower the free energy of the native folded state and consequently reduce the rate of misfolding [89].

On these basis, it was argued that the combination of a PRM along with a moiety able to modulate one or more PEs involved in prion pathogenesis might lead to discover effective MTLs. In this regard, the selected PRM was the 9-amino-6-chloro-2-methoxyacridine (compound 9, Figure 3F), a fragment present in the prototypical antiprion compound quinacrine, capable of interacting with prion proteins and modulating PrP^C misfolding [44–45,49,85,90–92].

Among PEs, increased OS is strongly associated with the development and progression of PrDs (Figure 3E) [93,94]. In light of this, a conspicuous number of antioxidant fragments (ascorbic and lipoic acid, polyphenols and carotenoids) have been proposed as beneficial against PrDs [95–97]. Among them, we focused on the lipoic acid (compound 10) fragment (Figure 3F) [98]. The endogenous antioxidant 10 was reported to be effective in an animal model [99] and in a prion infected patient [100],

and to be protective against protein aggregate toxicity [101]. The reduced form of 10 acts as an antioxidant by directly scavenging ROS, by reducing the oxidized form of other endogenous antioxidants, and by chelating transition metals, rendering such metals either redox inactive or facilitating their removal from the cell [102]. Compound 10 has also been proposed as a lead structure for designing MTLs for neurodegeneration [98,103–104]. Building on these premises, linked MTLs were designed by connecting a PRM to a lipoic acid moiety by an alkyl-amino chain [105]. Among the synthesized compounds, 11 (Figure 3F) showed a remarkable EC_{50} value of 150 nM and a concomitant low toxicity in the cell model of the disease. We were also able to demonstrate that, owing to the presence of the PRM and the lipoic acid fragment, 11 interacts with at least two of the multiple targets involved in prion pathology: it effectively inhibited PrP^{Sc} conversion in the ASA and reduced OS in a cellular context [105]. The reported *in vitro* profile makes this compound a promising candidate for further *in vivo* investigations and a valid starting point for the development of second-generation MTLs against PrD.

Theranostic small molecules

Brain amyloidogenic deposition is the main pathological hallmark of PrDs, which is believed to precede clinical symptoms by several years [106,107]. Therefore, imaging of fibrillar aggregates is particularly suitable to diagnose the onset of the disease in its early stage and monitor its progression [108]. On the other hand, as outlined above, amyloid fibrils have been shown to have therapeutic implications. Altogether, such findings raised captivating questions about the possibility of common strategies for monitoring the aggregation process and for therapeutic intervention as well [30]. In biomedicine an agent that combines diagnostic and therapeutic properties has been named theranostic [29–30,109]. This term emphasizes the simultaneous ability of a properly designed agent to allow the real-time assessment of the amount of drug reaching a pathological district and the visualization of molecular changes due to the therapeutic effects of the drug itself. Despite the theranostic research field is hard to investigate because of the sophisticated technology involved and the novel challenges posed, it offers concrete alternatives to the current paradigm of drug discovery. In this regard, examples of nanoparticles for improving diagnosis and therapy of AD already exist and have been recently reviewed by Andrieux and Couvreur [110].

In the last decade, great strides forward have been made in the field of molecular imaging of amyloid deposits *in vivo*. To this respect, MRI and PET are the most used techniques that already have clinical applications [111–113]. On the other hand, there is a growing interest in fluorescence spectroscopy as noninvasive alternatives for studying fibrillar aggregates. It is due to the fact that it is a versatile and sensitive method that lead to a rapid, inexpensive and nonradioactive imaging system for neurodegenerative disorders [114].

It is relevant to note that a critical feature for an ideal fluorescent probe is the ability to absorb and emit light in the near-infrared (NIR) region (600–900 nm), where tissue scattering and absorption is lowest. Although NIR imaging is so far limited to animal studies, it represents an attractive tool for early diagnosis due to its acceptable depth penetration and noninvasive operation [115–119].

Intriguingly, many molecules staining with high affinity scrapie fibrils turned out to be also capable of blocking their aggregation. To this respect, Iskikawa *et al.* provided two representative examples: a thioflavin (BTA-1) and a styrylbenzene (BSB) derivative (Figure 4) [120]. Both fluorescent probes, known to image A β plaques, were shown to stain prion fibrils *ex vivo* and to prevent abnormal PrP formation in a cellular model of TSEs. In addition, the incubation time of an experimental prion mouse model was prolonged when BSB was injected intravenously [120]. In this vein, amyloid ligands that showed concomitant capability of staining

abnormal prion deposits and inhibiting prion replication were recently reviewed [108,121]. Thus, it seems convincing that PrP^{Sc} binding compounds represent attractive candidates for monitoring the aggregation process and at the same time for therapeutic intervention.

In the light of these considerations, we aimed to design a small molecule able to label and detect PrP^{Sc} fibrils, and, ideally, to simultaneously block their aggregation [122].

We focused on fluorescent compounds endowed with proper features for its possible application *in vivo* including: to be able to change fluorescence properties upon binding to fibrils; to absorb and emit light in the NIR region; to modulate fibril aggregation; to have a small molecular size that enables the TL to cross the blood–brain barrier (BBB); and to possess low toxicity *in vivo*. Towards this goal, we developed a novel compound, (*E*)-6-methyl-4'-amino-2-styrylquinoline **12** (Figure 5) [122]. First, we investigated the ability of compound **12** to emit fluorescence in the NIR region by a native fluorescence study. Interestingly, we noted that the fluorescent spectra of the hydrochloride form of **12**, in its solid state, showed an emission maximum above 600 nm (NIR region). Thus, **12** can be viewed as a starting lead compound which can be easily chemically manipulated to enhance the spectral bathochromic shift, facilitating its use as a NIR sensor. Then, to assess its potential application as amyloid probe the emission spectra of **12** in the absence and presence of aggregated A β ₄₂ were recorded and a strong hypsochromic shift of the emission maximum (from 528 to 490 nm) was observed [122]. This behavior seemed related to a change in the environmental conditions, and it suggested that the binding of **12** to the amyloid fibrils took place. Furthermore, **12** acted as inhibitor of A β ₄₂ fibrilization towards in a thioflavin T (ThT)-based fluorimetric assay. To explore the labeling of PrP^{Sc} aggregates in living cells, fluorescent staining with **12**·HCl was carried out using a cell-based model of PrDs (ScGT1 cells). Fluorescent spots associated to prion fibrils were observed in scrapie-infected cells treated with **12**·HCl using a ThS filter, which is within the NIR optical window (Figure 5). Finally, the therapeutic antiprion potential of **12** was preliminarily assessed in the same ScGT1 cells (Figure 5) [122]. It showed a very low toxicity together with a remarkable activity (EC₅₀ of 0.5 μ M), which makes it more potent than GN8, an antiprion drug candidate [88]. In addition, **12** exhibited BBB permeability *in vitro* (parallel artificial membrane permeability assay test) demonstrating its value as CNS-directed agent [122].

Collectively, these data suggest that, with respect to the previously reported NIR amyloid sensors [115–118,123] and ruthenium complexes [124], **12** offers the advantage of a concomitant promising antifibrillar profile (*in vitro*

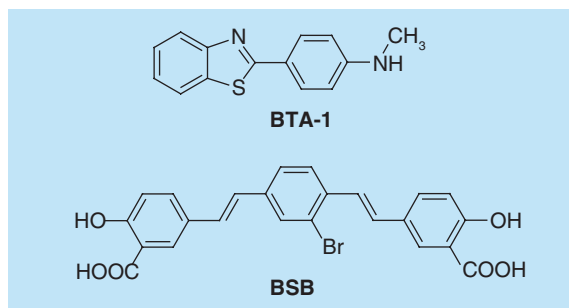


Figure 4. BTA-1 and BSB.

and in a cellular context). Furthermore, with regard to the widely used histological dyes Congo red and ThT, **12** shows a superior drug-likeness, thanks to its lower toxicity and higher BBB permeability.

In the light of these findings, **12** can be potentially useful to diagnose, deliver targeted therapy, and monitor response to therapy in PrDs. If its concomitant anti-aggregating profile and ability to bind fibrils will be confirmed *in vivo*, **12** might be the first small-molecule theranostic probe these illnesses.

Future perspective

Due to the continuous failures experienced, it can be argued that the existing model of developing drugs is no longer effective in neurodegeneration drug discovery, as well in some other therapeutic areas. The low innovation rate, in a context of limited drug efficacy and high incidence of adverse drug reactions, has been considered as the main culprit of the current predicament. Indeed over the years pharmaceutical companies have somehow moved away from innovative new-mode-of-action-products to less risky 'me-too' drugs [125]. But such a strategy might result in pipelines that lack truly innovative products and fails to generate the insights that can drive the field forward. Despite the gloomy outlook, many researchers are still plugging away, by expanding their drug discovery activities into the innovative companion diagnostic segment of personalized medicine [126].

The MTLs and TLs discussed in this review can be considered as truly innovative therapeutic tools. How-

ever, because no definitely clinical proof of concepts has been obtained, some would regard them as mere academic exercises. Thus, although a good deal of hype surrounds their potential of treating neurodegeneration, they might be deemed too unconventional for a realistic clinical application.

The history of science is filled with mavericks who refused to accept the prevailing theories and challenged the status quo. In the field of neurodegenerative diseases, those scientific mavericks included Stanley Prusiner, who admitted that initially "prion theory was greeted with great skepticism in most quarters and with outright disdain in others" [127].

Fostering scientific creativity has been recognized as a fundamental element of past and future success in the pharmaceutical industry [128]. In today's productivity crisis environment, out-of-the-box thinking is even more important. As underscored by John F Kennedy, when written in Chinese, the word 'crisis' is composed of two characters. One represents danger and the other represents opportunity. The dangers we face in developing innovative medicines are clear, but so are the opportunities. As academic researchers, we should focus more on the opportunities and be willing to take the risk of investing in less conventional tools, which, if successful, will have enormous public health benefits.

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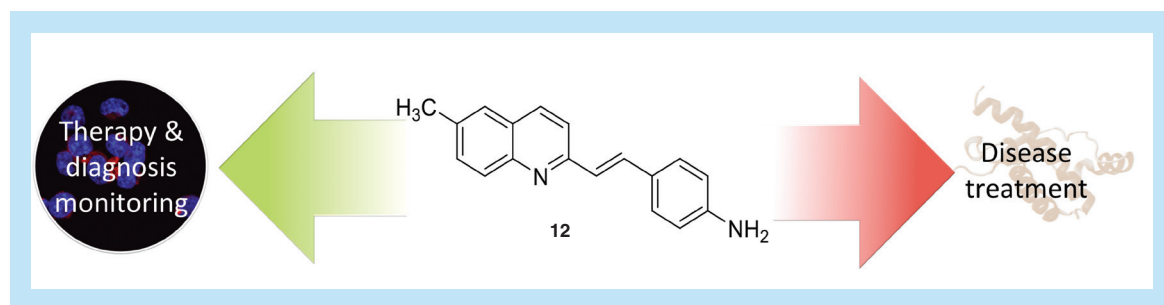


Figure 5. Diagnostic and therapeutic features of 12. Compound 12 has been shown to be able to stain prion fibrils and to inhibit prion replication in a cell-based model of prion diseases.

Executive summary**Innovative tools for prion diseases**

- The rational design of multitarget ligands (MTLs) and theranostic ligands (TLs) is a promising new strategy to combat prion diseases (PrDs).

Designing MTLs

- The mechanism underlying PrDs pathogenesis has been associated with a complex array of processes operating simultaneously and synergistically.
- Pathological processes involved are: PrP^C→PrP^{Sc} conversion; PrP^{Sc} aggregation; oxidative stress; unbalance of metal ions; and brain inflammation.
- Single molecules acting on multiple targets have a better potential to confront PrDs.
- MTLs are created by linking a fragment binding PrP^C with an antioxidant. Thus, a prion recognition motif and lipoic acid were joined by an alkylamino chain obtaining a compound that is the first rationally design MTL against PrDs.

Designing TLs

- TLs allow the simultaneously treatment of a disease (therapeutic) and visualization of molecular changes of a disease state (diagnostic).
- Styrylquinoline TL is able to prevent abnormal PrP^{Sc} formation in a prion cell culture model and to stain prion fibrils as well. Indeed, it combines therapeutic and diagnostic properties in a single molecule.

References

Papers of special note have been highlighted as:

• of interest; •• of considerable interest

- 1 Aguzzi A. Prion diseases of humans and farm animals: epidemiology, genetics, and pathogenesis. *J. Neurochem.* 97(6), 1726–1739 (2006).
- 2 Collinge J. Prion diseases of humans and animals: their causes and molecular basis. *Annu. Rev. Neurosci.* 24, 519–550 (2001).
- 3 Legname G, Laura Bolognesi M. Thematic issue: recent advances of biology and medicinal chemistry of prion protein and prions: on the road to therapeutics. *Curr. Top. Med. Chem.* 13(19), 2395–2396 (2013).
- 4 Soto C. Prion hypothesis: the end of the controversy? *Trends Biochem. Sci.* 36(3), 151–158 (2011).
- 5 Prusiner SB. Prions. *Proc. Natl. Acad. Sci. USA* 95(23), 13363–13383 (1998).
- 6 Kovacs GG, Budka H. Prion diseases: from protein to cell pathology. *Am. J. Pathol.* 172(3), 555–565 (2008).
- 7 Lloyd S, Mead S, Collinge J. Genetics of prion disease. *Top. Curr. Chem.* 305, 1–22 (2011).
- 8 Aguzzi A, Calella AM. Prions: protein aggregation and infectious diseases. *Physiol. Rev.* 89(4), 1105–1152 (2009).
- 9 Aguzzi A, Steele AD. Prion topology and toxicity. *Cell* 137(6), 994–996 (2009).
- 10 Dormont D. Prion diseases: pathogenesis and public health concerns. *FEBS Lett.* 529(1), 17–21 (2002).
- 11 The national CJD research and surveillance unit. www.cjd.ed.ac.uk
- 12 Prusiner SB. Cell biology. A unifying role for prions in neurodegenerative diseases. *Science* 336(6088), 1511–1513 (2012).
- Fascinating new perspective on neurodegenerative diseases pathogenesis.
- 13 Miller G. Could They all be prion diseases? *Science* 326(5958), 1337–1339 (2009).
- 14 Lahiri DK. Prions: a piece of the puzzle? *Science* 337(6099), 1172 (2012).
- 15 Jucker M, Walker LC. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature* 501(7465), 45–51 (2013).
- 16 Chen B, Thompson M, Louth J, Guo K. Prion chemical biology: on the road to therapeutics? *Curr. Top. Med. Chem.* 13(19), 2441–2464 (2013).
- 17 Pangalos MN, Schechter LE, Hurko O. Drug development for CNS disorders: strategies for balancing risk and reducing attrition. *Nat. Rev. Drug Discov.* 6(7), 521–532 (2007).
- 18 Enna SJ, Williams M. Challenges in the search for drugs to treat central nervous system disorders. *J. Pharmacol. Exp. Ther.* 329(2), 404–411 (2009).
- 19 Markou A, Chiamulera C, Geyer MA, Tricklebank M, Steckler T. Removing obstacles in neuroscience drug discovery: the future path for animal models. *Neuropsychopharmacology* 34(1), 74–89 (2009).
- 20 Becker RE, Greig NH. Increasing the success rate for Alzheimer's disease drug discovery and development. *Expert Opin. Drug Discov.* 7(4), 367–370 (2012).
- 21 Bolognesi ML, Melchiorre C, Van Der Schyf CJ, Youdim M. Discovery of multi-target agents for neurological diseases via ligand design. In: *Designing Multi-Target Drugs*. Morphy JR, Harris CJ (Eds). The Royal Society of Chemistry, London, UK, 290–315 (2012).
- 22 Chen X, Decker M. Multi-target compounds acting in the central nervous system designed from natural products. *Curr. Med. Chem.* 20(13), 1673–1685 (2013).
- 23 Cavalli A, Bolognesi ML, Minarini A *et al.* Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.* 51(3), 347–372 (2008).
- 24 Geldenhuys WJ, Youdim MB, Carroll RT, Van Der Schyf CJ. The emergence of designed multiple ligands for neurodegenerative disorders. *Prog. Neurobiol.* 94(4), 347–359 (2011).
- 25 Leon R, Garcia AG, Marco-Contelles J. Recent advances in the multitarget-directed ligands approach for the treatment

- of Alzheimer's disease. *Med. Res. Rev.* 33(1), 139–189 (2013).
- 26 Munoz-Torrero D. Complexity against complexity: multitarget drugs. *Curr. Med. Chem.* 20(13), 1621–1622 (2013).
- 27 Cavalli A, Bolognesi ML. Multitargeted drugs for treatment of alzheimer's disease. In: *Polypharmacology in Drug Discovery*. Peters JU (Ed.). John Wiley & Sons, Inc., NJ, USA, 441–458 (2012).
- 28 Kelkar SS, Reineke TM. Theranostics: combining imaging and therapy. *Bioconjug. Chem.* 22(10), 1879–1903 (2011).
- 29 Lammers T, Aime S, Hennink WE, Storm G, Kiessling F. Theranostic nanomedicine. *Acc. Chem. Res.* 44(10), 1029–1038 (2011).
- **Excellent review highlighting the potential of theranostics in biomedicine.**
- 30 Bolognesi ML. Amyloid chemical probes and theranostics: steps toward personalized medicine in neurodegenerative diseases. In: *Medicinal Chemistry Approaches to Personalized Medicine*. Lackey K, Roth BD (Eds.). Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 211–226 (2013).
- 31 Soto C, Satani N. The intricate mechanisms of neurodegeneration in prion diseases. *Trends Mol. Med.* 17(1), 14–24 (2011).
- 32 Trevitt CR, Collinge J. A systematic review of prion therapeutics in experimental models. *Brain* 129(Pt 9), 2241–2265 (2006).
- 33 Gavrin LK, Denny RA, Saiah E. Small molecules that target protein misfolding. *J. Med. Chem.* 55(24), 10823–10843 (2012).
- **Comprehensive update on small molecules that modulate aberrant proteins from a medicinal chemistry point of view.**
- 34 Kranjc A, Bongarzone S, Rossetti G *et al.* Docking ligands on protein surfaces: the case study of prion protein. *J. Chem. Theory Comput.* 5(9), 2565–2573 (2009).
- 35 Brown DR. Prion protein peptides: optimal toxicity and peptide blockade of toxicity. *Mol. Cell Neurosci.* 15(1), 66–78 (2000).
- 36 Perez M, Sadqi M, Munoz V, Avila J. Inhibition by aplidine of the aggregation of the prion peptide PrP 106–126 into beta-sheet fibrils. *Biochim. Biophys. Acta* 1639(2), 133–139 (2003).
- 37 Vilches S, Vergara C, Nicolas O *et al.* Neurotoxicity of prion peptides mimicking the central domain of the cellular prion protein. *PLoS ONE* 8(8), e70881 (2013).
- 38 Wang X, He L, Zhao C, Du W, Lin J. Gold complexes inhibit the aggregation of prion neuropeptides. *J. Biol. Inorg. Chem.* 18(7), 767–778 (2013).
- 39 Wang Y, Feng L, Zhang B *et al.* Palladium complexes affect the aggregation of human prion protein PrP106–126. *Inorg. Chem.* 50(10), 4340–4348 (2011).
- 40 Keunhong J, Junghun S, Woo Young C, Young Sik K, Dongwook K, Tae Yeon L. New methodology for estimation of the prion protein 106–126 amyloid aggregation. *Bull. Korean Chem. Soc.* 31(4), 1029–1030 (2010).
- 41 Baral PK, Swayampakula M, Rout MK *et al.* Structural basis of prion inhibition by phenothiazine compounds. *Structure* 22(2), 291–303 (2014).
- **Illuminating article on phenothiazines antiprion mechanism of action.**
- 42 Staderini M, Legname G, Bolognesi ML, Menendez JC. Modulation of prion by small molecules: from monovalent to bivalent and multivalent ligands. *Curr. Top. Med. Chem.* 13(19), 2491–2503 (2013).
- 43 Horton DA, Bourne GT, Smythe ML. Exploring privileged structures: the combinatorial synthesis of cyclic peptides. *J. Comput. Aided Mol. Des.* 16(5–6), 415–430 (2002).
- 44 Bolognesi ML, Ai Tran HN, Staderini M *et al.* Discovery of a class of diketopiperazines as antiprion compounds. *ChemMedChem* 5(8), 1324–1334 (2010).
- 45 Bongarzone S, Bolognesi ML. The concept of privileged structures in rational drug design: focus on acridine and quinoline scaffolds in neurodegenerative and protozoan diseases. *Expert Opin. Drug Discov.* 6(3), 251–268 (2011).
- 46 Tran HN, Bongarzone S, Carloni P, Legname G, Bolognesi ML. Synthesis and evaluation of a library of 2,5-bisdiamino-benzoquinone derivatives as probes to modulate protein–protein interactions in prions. *Bioorg. Med. Chem. Lett.* 20(6), 1866–1868 (2010).
- 47 Azriel R, Gazit E. Analysis of the minimal amyloid-forming fragment of the islet amyloid polypeptide. An experimental support for the key role of the phenylalanine residue in amyloid formation. *J. Biol. Chem.* 276(36), 34156–34161 (2001).
- 48 Bartolini M, Bertucci C, Bolognesi ML, Cavalli A, Melchiorre C, Andrisano V. Insight into the kinetic of amyloid beta (1–42) peptide self-aggregation: elucidation of inhibitors' mechanism of action. *Chembiochem* 8(17), 2152–2161 (2007).
- 49 Bongarzone S, Tran HN, Cavalli A *et al.* Parallel synthesis, evaluation, and preliminary structure-activity relationship of 2,5-diamino-1,4-benzoquinones as a novel class of bivalent antiprion compound. *J. Med. Chem.* 53(22), 8197–8201 (2010).
- 50 Colby DW, Zhang Q, Wang S *et al.* Prion detection by an amyloid seeding assay. *Proc. Natl Acad. Sci. USA* 104(52), 20914–20919 (2007).
- 51 Petrelli A, Valabrega G. Multitarget drugs: the present and the future of cancer therapy. *Expert Opin. Pharmacother.* 10(4), 589–600 (2009).
- 52 Millan MJ. Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol. Ther.* 110(2), 135–370 (2006).
- 53 Wong EH, Tarazi FI, Shahid M. The effectiveness of multi-target agents in schizophrenia and mood disorders: relevance of receptor signature to clinical action. *Pharmacol. Ther.* 126(2), 173–185 (2010).
- 54 Youdim MB, Kupersmidt L, Amit T, Weinreb O. Promises of novel multi-target neuroprotective and neurorestorative drugs for Parkinson's disease. *Parkinsonism Relat. Disord.* 20(Suppl. 1), S132–S136 (2014).
- 55 Youdim MB. Multi target neuroprotective and neurorestorative anti-Parkinson and anti-Alzheimer drugs ladostigil and m30 derived from rasagiline. *Exp. Neurobiol.* 22(1), 1–10 (2013).

- 56 Morphy R. Selective multitargeted drugs. In: *Polypharmacology in Drug Discovery*, Peters J-U (Ed.). John Wiley & Sons, Inc., NJ, USA, 245–262 (2012).
- 57 Morphy JR, Harris CJ. Designing multi-target drugs. In: *RSC Drug Discovery*. Royal Society of Chemistry, Cambridge, UK (2012).
- 58 Morphy R, Rankovic Z. The physicochemical challenges of designing multiple ligands. *J. Med. Chem.* 49(16), 4961–4970 (2006).
- 59 Das N, Dhanawat M, Dash B, Nagarwal RC, Shrivastava SK. Codrug: an efficient approach for drug optimization. *Eur. J. Pharm. Sci.* 41(5), 571–588 (2010).
- 60 Portoghese PS. From models to molecules: opioid receptor dimers, bivalent ligands, and selective opioid receptor probes. *J. Med. Chem.* 44(14), 2259–2269 (2001).
- 61 Bhushan RG, Sharma SK, Xie Z, Daniels DJ, Portoghese PS. A bivalent ligand (KDN-21) reveals spinal delta and kappa opioid receptors are organized as heterodimers that give rise to delta(1) and kappa(2) phenotypes. Selective targeting of delta-kappa heterodimers. *J. Med. Chem.* 47(12), 2969–2972 (2004).
- 62 Morphy R, Kay C, Rankovic Z. From magic bullets to designed multiple ligands. *Drug Discov. Today* 9(15), 641–651 (2004).
- 63 Morphy R, Rankovic Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* 48(21), 6523–6543 (2005).
- 64 Morphy R, Rankovic Z. Fragments, network biology and designing multiple ligands. *Drug Discov. Today* 12(3–4), 156–160 (2007).
- 65 Morphy R, Rankovic Z. Designing multiple ligands – medicinal chemistry strategies and challenges. *Curr. Pharm. Des.* 15(6), 587–600 (2009).
- 66 Caughey B, Baron GS. Prions and their partners in crime. *Nature* 443(7113), 803–810 (2006).
- 67 Legname G, Baskakov IV, Nguyen HO *et al.* Synthetic mammalian prions. *Science* 305(5684), 673–676 (2004).
- 68 Arlt S, Kontush A, Zerr I *et al.* Increased lipid peroxidation in cerebrospinal fluid and plasma from patients with Creutzfeldt–Jakob disease. *Neurobiol. Dis.* 10(2), 150–156 (2002).
- 69 Klamt F, Dal-Pizzol F, Conte Da Frota ML Jr *et al.* Imbalance of antioxidant defense in mice lacking cellular prion protein. *Free Radic. Biol. Med.* 30(10), 1137–1144 (2001).
- 70 Freixes M, Rodriguez A, Dalfo E, Ferrer I. Oxidation, glycoxidation, lipoxidation, nitration, and responses to oxidative stress in the cerebral cortex in Creutzfeldt–Jakob disease. *Neurobiol. Aging* 27(12), 1807–1815 (2006).
- 71 Milhavel O, McMahon HE, Rachidi W *et al.* Prion infection impairs the cellular response to oxidative stress. *Proc. Natl Acad. Sci. USA* 97(25), 13937–13942 (2000).
- 72 Pamplona R, Naudi A, Gavin R *et al.* Increased oxidation, glycoxidation, and lipoxidation of brain proteins in prion disease. *Free Radic. Biol. Med.* 45(8), 1159–1166 (2008).
- 73 Singh N, Singh A, Das D, Mohan ML. Redox control of prion and disease pathogenesis. *Antioxid. Redox Signal* 12(11), 1271–1294 (2010).
- 74 Lehmann S. Metal ions and prion diseases. *Curr. Opin. Chem. Biol.* 6(2), 187–192 (2002).
- 75 Perry VH, Cunningham C, Boche D. Atypical inflammation in the central nervous system in prion disease. *Curr. Opin. Neurol.* 15(3), 349–354 (2002).
- 76 Mallucci G, Collinge J. Rational targeting for prion therapeutics. *Nat. Rev. Neurosci.* 6(1), 23–34 (2005).
- 77 Nicoll AJ, Collinge J. Preventing prion pathogenicity by targeting the cellular prion protein. *Infect. Disord. Drug Targets* 9(1), 48–57 (2009).
- 78 Stewart LA, Rydzewska LH, Keogh GF, Knight RS. Systematic review of therapeutic interventions in human prion disease. *Neurology* 70(15), 1272–1281 (2008).
- 79 Caughey B, Caughey WS, Kocisko DA, Lee KS, Silveira JR, Morrey JD. Prions and transmissible spongiform encephalopathy (TSE) chemotherapeutics: a common mechanism for anti-TSE compounds? *Acc. Chem. Res.* 39(9), 646–653 (2006).
- 80 Krammer C, Vorberg I, Scharzl HM, Gilch S. Therapy in prion diseases: from molecular and cellular biology to therapeutic targets. *Infect. Disord. Drug Targets* 9(1), 3–14 (2009).
- 81 Ludewigs H, Zuber C, Vana K, Nikles D, Zerr I, Weiss S. Therapeutic approaches for prion disorders. *Expert Rev. Anti Infect. Ther.* 5(4), 613–630 (2007).
- 82 Sim VL, Caughey B. Recent advances in prion chemotherapeutics. *Infect. Disord. Drug Targets* 9(1), 81–91 (2009).
- 83 Wisniewski T, Sigurdsson EM. Therapeutic approaches for prion and Alzheimer's diseases. *FEBS J.* 274(15), 3784–3798 (2007).
- 84 Nunziante M, Gilch S, Scharzl HM. Prion diseases: from molecular biology to intervention strategies. *Chembiochem* 4(12), 1268–1284 (2003).
- 85 Cope H, Mutter R, Heal W *et al.* Synthesis and SAR study of acridine, 2-methylquinoline and 2-phenylquinazoline analogues as antiprion agents. *Eur. J. Med. Chem.* 41(10), 1124–1143 (2006).
- 86 Guo K, Mutter R, Heal W *et al.* Synthesis and evaluation of a focused library of pyridine dicarbonitriles against prion disease. *Eur. J. Med. Chem.* 43(1), 93–106 (2008).
- 87 Heal W, Thompson MJ, Mutter R, Cope H, Louth JC, Chen B. Library synthesis and screening: 2,4-diphenylthiazoles and 2,4-diphenyloxazoles as potential novel prion disease therapeutics. *J. Med. Chem.* 50(6), 1347–1353 (2007).
- 88 Kuwata K, Nishida N, Matsumoto T *et al.* Hot spots in prion protein for pathogenic conversion. *Proc. Natl Acad. Sci. USA* 104(29), 11921–11926 (2007).
- 89 Rossetti G, Bongarzone S, Carloni P. Computational studies on the prion protein. *Curr. Top. Med. Chem.* 13(19), 2419–2431 (2013).
- 90 Macedo B, Kaschula CH, Hunter R *et al.* Synthesis and antiprion activity evaluation of aminoquinoline analogues. *Eur. J. Med. Chem.* 45(11), 5468–5473 (2010).
- 91 May BC, Fafarman AT, Hong SB *et al.* Potent inhibition of scrapie prion replication in cultured cells by bis-acridines. *Proc. Natl Acad. Sci. USA* 100(6), 3416–3421 (2003).

- 92 Dollinger S, Lober S, Klingenstein R, Korth C, Gmeiner P. A chimeric ligand approach leading to potent antiprion active acridine derivatives: design, synthesis, and biological investigations. *J. Med. Chem.* 49(22), 6591–6595 (2006).
- 93 Choi SI, Ju WK, Choi EK *et al.* Mitochondrial dysfunction induced by oxidative stress in the brains of hamsters infected with the 263 K scrapie agent. *Acta Neuropathol.* 96(3), 279–286 (1998).
- 94 Milhavel O, Lehmann S. Oxidative stress and the prion protein in transmissible spongiform encephalopathies. *Brain Res. Brain Res. Rev.* 38(3), 328–339 (2002).
- 95 Gilgun-Sherki Y, Melamed E, Offen D. Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. *Neuropharmacology* 40(8), 959–975 (2001).
- 96 Sinatra ST, Demarco J. Free radicals, oxidative stress, oxidized low density lipoprotein (LDL), and the heart: antioxidants and other strategies to limit cardiovascular damage. *Conn. Med.* 59(10), 579–588 (1995).
- 97 Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr. Neuropharmacol.* 7(1), 65–74 (2009).
- 98 Bolognesi ML, Minarini A, Tumiatti V, Melchiorre C. Lipoic acid, a lead structure for multi-target-directed drugs for neurodegeneration. *Mini Rev. Med. Chem.* 6(11), 1269–1274 (2006).
- 99 Suh JH, Moreau R, Heath SH, Hagen TM. Dietary supplementation with (R)-alpha-lipoic acid reverses the age-related accumulation of iron and depletion of antioxidants in the rat cerebral cortex. *Redox Rep.* 10(1), 52–60 (2005).
- 100 Drisko JA. The use of antioxidants in transmissible spongiform encephalopathies: a case report. *J. Am. Coll. Nutr.* 21(1), 22–25 (2002).
- 101 Zhang L, Xing GQ, Barker JL *et al.* Alpha-lipoic acid protects rat cortical neurons against cell death induced by amyloid and hydrogen peroxide through the Akt signalling pathway. *Neurosci. Lett.* 312(3), 125–128 (2001).
- 102 Smith AR, Shenvi SV, Widlansky M, Suh JH, Hagen TM. Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Curr. Med. Chem.* 11(9), 1135–1146 (2004).
- 103 Rosini M, Andrisano V, Bartolini M *et al.* Rational approach to discover multipotent anti-Alzheimer drugs. *J. Med. Chem.* 48(2), 360–363 (2005).
- 104 Bolognesi ML, Cavalli A, Bergamini C *et al.* Toward a rational design of multitarget-directed antioxidants: merging mequinone and lipoic acid molecular frameworks. *J. Med. Chem.* 52(23), 7883–7886 (2009).
- 105 Bongarzone S, Tran HN, Cavalli A *et al.* Hybrid lipoic acid derivatives to attack prion disease on multiple fronts. *ChemMedChem* 6(4), 601–605 (2011).
- **First report of a multitarget ligand against prion diseases.**
- 106 Tateishi J, Kitamoto T. Developments in diagnosis for prion diseases. *Br. Med. Bull.* 49(4), 971–979 (1993).
- 107 Schulz-Schaeffer WJ, Tschoke S, Kranefuss N *et al.* The paraffin-embedded tissue blot detects PrP(Sc) early in the incubation time in prion diseases. *Am. J. Pathol.* 156(1), 51–56 (2000).
- 108 Aulic S, Bolognesi ML, Legname G. Small-molecule theranostic probes: a promising future in neurodegenerative diseases. *Int. J. Cell Biol.* 2013,150952 (2013).
- 109 Terreno E, Uggeri F, Aime S. Image guided therapy: the advent of theranostic agents. *J. Control. Release* 161(2), 328–337 (2012).
- 110 Andrieux K, Couvreur P. Nanomedicine as a promising approach for the treatment and diagnosis of brain diseases: the example of Alzheimer's disease. *Ann. Pharm. Fr.* 71(4), 225–233 (2013).
- 111 Raji CA, Becker JT, Tsopelas ND *et al.* Characterizing regional correlation, laterality and symmetry of amyloid deposition in mild cognitive impairment and Alzheimer's disease with Pittsburgh compound B. *J. Neurosci. Methods* 172(2), 277–282 (2008).
- 112 Rowe CC, Ackerman U, Browne W *et al.* Imaging of amyloid beta in Alzheimer's disease with 18F-BAY94–9172, a novel PET tracer: proof of mechanism. *Lancet Neurol.* 7(2), 129–135 (2008).
- 113 Choi SR, Golding G, Zhuang Z *et al.* Preclinical properties of 18F-AV-45: a PET agent for Abeta plaques in the brain. *J. Nucl. Med.* 50(11), 1887–1894 (2009).
- 114 Bertocini CW, Celej MS. Small molecule fluorescent probes for the detection of amyloid self-assembly *in vitro* and *in vivo*. *Curr. Protein Pept. Sci.* 12(3), 205–220 (2011).
- **Accurate review of fluorescent small molecules capable of detecting amyloid aggregates.**
- 115 Hintersteiner M, Enz A, Frey P *et al.* *In vivo* detection of amyloid-beta deposits by near-infrared imaging using an oxazine-derivative probe. *Nat. Biotechnol.* 23(5), 577–583 (2005).
- **Description of a near-infrared probe able to detect amyloid plaques *in vivo*.**
- 116 Ran C, Xu X, Raymond SB *et al.* Design, synthesis, and testing of difluoroboron-derivatized curcumins as near-infrared probes for *in vivo* detection of amyloid-beta deposits. *J. Am. Chem. Soc.* 131(42), 15257–15261 (2009).
- 117 Schmidt A, Pahnke J. Efficient near-infrared *in vivo* imaging of amyloid-beta deposits in Alzheimer's disease mouse models. *J. Alzheimers Dis.* 30(3), 651–664 (2012).
- 118 Nesterov EE, Skoch J, Hyman BT, Klunk WE, Bacskai BJ, Swager TM. *In vivo* optical imaging of amyloid aggregates in brain: design of fluorescent markers. *Angew. Chem. Int. Ed. Engl.* 44(34), 5452–5456 (2005).
- 119 Weissleder R, Pittet MJ. Imaging in the era of molecular oncology. *Nature* 452(7187), 580–589 (2008).
- 120 Ishikawa K, Doh-Ura K, Kudo Y *et al.* Amyloid imaging probes are useful for detection of prion plaques and treatment of transmissible spongiform encephalopathies. *J. Gen. Vir.* 85(6), 1785–1790 (2004).
- 121 Teruya K, Doh-Ura K. Amyloid-binding compounds and their antiprion potency. *Curr. Top. Med. Chem.* 13(19), 2522–2532 (2013).

- 122 Staderini M, Aulic S, Bartolini M *et al.* A fluorescent styrylquinoline with combined therapeutic and diagnostic activities against Alzheimer's and prion diseases. *ACS Med. Chem. Lett.* 4(2), 225–229 (2012).
- 123 Raymond SB, Skoch J, Hills ID, Nesterov EE, Swager TM, Bacskai BJ. Smart optical probes for near-infrared fluorescence imaging of Alzheimer's disease pathology. *Eur. J. Nucl. Med. Mol. Imaging* 35(Suppl. 1), S93–S98 (2008).
- 124 Cook NP, Ozbil M, Katsampes C, Prabhakar R, Marti AA. Unraveling the photoluminescence response of light-switching ruthenium(II) complexes bound to amyloid-beta. *J. Am. Chem. Soc.* 135(29), 10810–10816 (2013).
- 125 Olivier C, Williams-Jones B, Godard B, Mikalson B, Ozdemir V. Personalized medicine, bioethics and social responsibilities: re-thinking the pharmaceutical industry to remedy inequities in patient care and international health. *Curr. Pharmacogenomics Person. Med.* 6(2), 108–120 (2008).
- 126 Merck and Luminex Corporation Enter Agreement to Develop Companion Diagnostic to Support Investigational BACE inhibitor Clinical Development Program for Alzheimer's Disease. www.merck.com/licensing/our-partnership/luminex-partnership.html
- 127 Prusiner SB. Detecting mad cow disease. *Sci. Am.* 291(1), 86–93 (2004).
- 128 Cuatrecasas P. Drug discovery in jeopardy. *J. Clin. Invest.* 116(11), 2837–2842 (2006).