MedChemComm

Cite this: *Med. Chem. Commun.*, 2011, **2**, 60

www.rsc.org/medchemcomm

CONCISE ARTICLE

A rational design to create hybrid β-sheet breaker peptides to inhibit aggregation and toxicity of amyloid-β†

Ilona B. Bruinsma,*[ab] Anna Karawajczyk,[c] Gijs Schaftenaar,[c] Robert M. W. de Waal,[d] Marcel M. Verbeek[ab] and Floris L. van Delft[e]

Received 11th November 2010, Accepted 29th November 2010
DOI: 10.1039/c0md00213e

Alzheimer's disease is characterized by deposits of the amyloid β protein (Aβ) in the form of senile plaques and cerebral amyloid angiopathy. Deposition of Aβ into these pathological lesions is directed by step-wise aggregation of Aβ into oligomers, protofibrils and mature fibrils. Currently, all therapies are purely symptom-relieving, and an actual treatment or prevention of AD is still lacking. Since aggregated forms of Aβ are particularly neurotoxic, interference with the process of Aβ aggregation is a long-envisioned target for therapy. Based on the knowledge that both sulfated (macro)molecules and small synthetic peptides interfere with Aβ aggregation, we developed hybrid ligands to target Aβ fibrillization by a combination of peptide-peptide and sulfate-peptide interactions. A series of peptides, modified at the N-terminus with sulfated linkers, was successfully prepared by solid phase synthesis. The hybrid ligands were tested using a viability assay and an aggregation assay. Molecular modeling was applied to explain the binding of the hybrid ligands to $A\beta_{42}$. The hybrid ligands that we designed, synthesized and evaluated were found to be non-toxic to cells but displayed negligible inhibition of Aβ fibrillization and Aβ-mediated cytotoxicity compared to the beta-sheet breaker peptides known today. Further molecular modeling simulations suggested that the hybrid ligands were incorporated into the β-sheet structure of Aβ aggregates, indicating that the hybrid ligands may bind to Aβ but are unable to inhibit further aggregation. Optimization of the hybrid ligands by reducing hydrogen bond interactions of the ligand with following Aβ proteins might result in ligands, with improved binding to one Aβ protein, that could potentially disrupt further β-sheet formation. This in turn may reduce toxicity of Aβ.

Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by a progression from episodic memory problems to a slow global decline of cognitive function that leaves patients with end-stage AD bed-ridden and dependent on custodial care. It affects 10% of the people over the age of 65, and accounts for approximately 50% of all patients with dementia.[1] The current standard of care for mild to moderate AD includes treatment with acetylcholinesterase inhibitors to improve cognitive function. In addition, the common non-cognitive neuropsychiatric symptoms of AD (such as mood disorder, agitation, and psychosis) often require extra medication.[2] However, these treatments are purely symptom-relieving, and an actual treatment or prevention of AD is still lacking.

Histopathological features of AD are extensive deposition of the amyloid β protein (Aβ) in senile plaques (SPs) in the cerebral cortex as well as in the cerebrovascular wall (cerebral amyloid angiopathy, CAA). This deposition is a result from the conversion of α-helical or random coil Aβ to β-sheet, which facilitates self-aggregation of normally soluble Aβ into dimers, trimers, oligomers, protofibrils and eventually mature insoluble fibrils.[3] In addition, the formation of β-sheet-rich aggregates has been reported to be toxic towards various cultured cerebral cells, including neurons, cerebrovascular smooth muscle cells and human brain pericytes.[4–7] Taken together, this suggests that Aβ might be involved in the pathogenesis of AD.

An attractive therapeutic strategy for AD is to block the early steps of misfolding and aggregation of the soluble Aβ by using small molecule drugs. If the peptide interactions are the same in

[a]*Radboud University Nijmegen Medical Centre, Donders Institute for Brain, Cognition and Behaviour, Centre for Neuroscience, Department of Neurology and Alzheimer Centre, 830 LGEM, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. E-mail: i.bruinsma@neuro.umcn.nl; Fax: +31-243668754; Tel: +31-243668965*
[b]*Radboud University Nijmegen Medical Centre, Donders Institute for Brain, Cognition and Behaviour, Centre for Neuroscience, Department of Laboratory Medicine, Laboratory of Genetic, Endocrine and Metabolic Diseases, The Netherlands*
[c]*Centre for Molecular and Biomolecular Informatics, Radboud University, Nijmegen, The Netherlands*
[d]*Radboud University Nijmegen Medical Centre, Department of Pathology, Nijmegen, the Netherlands*
[e]*Institute for Molecules and Materials, Radboud University Nijmegen, Synthetic Organic Chemistry, Nijmegen, The Netherlands*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c0md00213e

oligomers and in larger fibrils, then such molecules could inhibit both the formation of toxic oligomers and of fibrils. To this end, several research groups have designed "β-sheet breakers" (BSB).[8,9] BSB are based on peptide sequences that specifically interfere with β-sheets within Aβ. Two sets of peptides were effective BSB, namely the peptides LPFFD (or its analogues LVFFA or LPYFD) and KLVFF that bind to a region between amino acids 16 and 22 of Aβ.[8,10,11] These compounds inhibit Aβ-mediated neurotoxicity[10,12] and Aβ deposition *in vivo* and improve behavioral deficiency induced by Aβ deposition.[10,12,13] However, a reasonable molar excess compared to the Aβ protein is needed to achieve such a result.[10,14,15]

It is known that small sulfated and sulfonated molecules have high binding affinity with Aβ, since they are designed to interfere with glycosaminoglycan/Aβ interaction.[19] The binding site on Aβ for GAGs likely resides within the cluster of basic amino acids 13–16 of Aβ,[20] although it remains possible that other domains within Aβ interact with GAGs as well.[21] Thus, this might indicate that the binding site on Aβ for the small anionic sulfates and sulfonates also resides within the same cluster of amino acids.

Covalent linking of two separate ligands to give so-called hybrid ligands is a powerful general concept to enhance binding affinity and improve the biological activity compared to the separate molecules, a strategy that has successfully been used in medicinal chemistry in the past years.[16–18] Increasing the binding affinity of a BSB peptide to Aβ might decrease the molar excess needed to affect Aβ aggregation, toxicity and deposition. Considering the relative positions of BSB peptides and GAG binding to Aβ (amino acids 16–22 and 13–16 respectively),[8,10,20,22,23] it occurred to us that the covalent attachment of (poly)sulfates with the BSB peptide by a certain linker would possibly lead to a hybrid with improved binding characteristics and, thereby, possibly improved characteristics with respect to inhibition of Aβ fibrillization and Aβ-mediated cytotoxicity. Hence, we synthesized Aβ ligands that combine pentapeptide-Aβ interaction and sulfate-Aβ interaction and evaluated their effects on Aβ aggregation and Aβ-mediated cytotoxicity relative to the separate ligands.

Since BSB peptide KLVFF contains the basic amino acid lysine (K), which would result in a zwitterionic structure upon monosulfonylation, BSB peptide LPFFD, lacking a basic amino acid, was chosen as starting point of our hybrid ligands. The synthetic route towards several hybrid ligands (**3**, **4**, **5a–5d** and **6a–6d**) is shown in Fig. 1, and is based on standard Fmoc-based solid phase synthesis and introduction of a variety of alcohols at the N-terminus of the pentapeptide, *via* carbamate or urea functionality. Because cleavage of $C_5H_{10}O_4$-LPFFD-OH (**5d** (L-Pro) and **5d′** (D-Pro)) from the Breipohl resin did not result in the desired compound, likely caused by the harsh cleavage conditions, a 2-chlorotrityl resin was used instead. The milder conditions for cleavage from the 2-chlorotrityl resin successfully afforded $C_5H_{10}O_4$-LPFFD-OH (**5d** and **5d′**). After cleavage from the resin, sulfate groups were introduced by sulfonylation of the alcohol groups under the action of sulfurtrioxide-amine complex. Using HPLC, a purity of >95% was achieved for all synthetic sequences. In addition to the hybrid ligands shown in Fig. 1, as a control peptide Ac-LPFFD-NH_2 (**7**) was also synthesized. Ac-LPFFD-NH_2 (**7**) is known in literature as iAβ5p and is shown to reduce amyloid plaque formation in a transgenic mouse model.[14] In our study, it served as a control to our hybrid ligands in the biological assays.

Two kinds of assays were used to determine the biological activity of the hybrid ligands. First of all, hybrid ligands were screened for *in vitro* activity in inhibition of amyloid fibril formation. Fibril formation was quantified with a fluorescence assay based on the specific binding of thioflavin T (Th-T) to β-sheet amyloid aggregates.[24] By binding to the β-sheets in Aβ aggregates, a fluorescent signal is produced which is proportional to the amount of fibrils formed. The effect of the hybrid ligands on β-sheet formation of $Aβ_{42}$ in the first 48 h of incubation was analyzed and compared to the inhibitory effect of Ac-LPFFD-NH_2 (**7**) alone. In earlier studies (unpublished data) it became apparent that co-incubation of (monomeric) Aβ with Ac-LPFFD-NH_2 (**7**) in a molar ratio of 1 : 20 resulted in approximately 50% inhibition of the aggregation of Aβ. Therefore, we have used this molar ratio to study the hybrid ligands in the aggregation assay. Secondly, toxicity of Aβ in cell culture has been reported to be related to the formation of β-sheet-rich aggregates[6] and has been used in several studies to screen diverse compounds to prevent amyloid neurotoxicity. Degeneration of smooth muscle cells (SMCs) and pericytes by Aβ (aggregation) is a prominent feature of CAA. We, therefore, developed a model of Aβ-mediated degeneration of cultured human brain pericytes (HBPs) and SMCs.[25,26] These cells were isolated from human brain capillaries and leptomeningeal vessels, respectively, from control and AD brains obtained at autopsy. In this model the aggregation of wild-type $Aβ_{1–42}$, is cytotoxic for HBP and SMC cultures.[5] The effect of the hybrid ligands on $Aβ_{42}$-mediated cell death after 6 days of incubation was analyzed and compared to the inhibitory effect of Ac-LPFFD-NH_2 (**7**) alone. In earlier studies (unpublished data) it became apparent that co-incubation of (monomeric) Aβ with Ac-LPFFD-NH_2 (**7**) in a molar ratio of 1 : 5 resulted in approximately 50% inhibition of the cytotoxicity of Aβ. Therefore, we have used this molar ratio to study the hybrid ligands in the viability assay.

Co-incubation of $Aβ_{42}$ with Ac-LPFFD-NH_2 (**7**) resulted in an inhibition of the toxicity and aggregation of $Aβ_{42}$ (Fig. 2), which confirms earlier research.[10] However, no significant effect was found of either hybrid ligand both on toxicity and aggregation of $Aβ_{42}$. In Table 1 (supporting materials) an overview of the hybrid ligands and their effects on aggregation and toxicity of $Aβ_{42}$ is given. Fig. 2 shows a representative figure for all hybrid ligands. Co-incubation of $Aβ_{42}$ with Ac-LPFFD-NH_2 (**7**) in a molar ratio of 1 : 20 results in 66% decrease in fluorescent signal ($p < 0.01$; Fig. 2A), *i.e.* Ac-LPFFD-NH_2 (**7**) seems to inhibit the fibril formation of $Aβ_{42}$, which confirms earlier research.[27] However, co-incubation of $Aβ_{42}$ with $C_5H_{10}O_4$-LPFFD-OH (**5d**) or the bissulfonylated analogue $C_5H_8Na_2O_{10}S_2$-LPFFD-OH (**6d**) in a molar ratio of 1 : 20 resulted in a fluorescent signal comparable to the signal of $Aβ_{42}$ alone (Fig. 2A). Incubation of buffer alone or the compounds themselves did not result in a fluorescent signal. In addition, a significant difference was observed in cell death between HBPs treated with 10 μM $Aβ_{42}$ either alone (25% cell death) or with Ac-LPFFD-NH_2 (**7**; 11.3% cell death; $p < 0.001$; Fig. 2B) in a molar ratio of 1 : 5 for 6 days, which also confirms earlier research.[10,12] However, again no significant effect was observed when co-incubating $Aβ_{42}$ with **5d** or **6d** in a molar ratio of 1 : 5 for 6 days compared to incubation with $Aβ_{42}$ alone

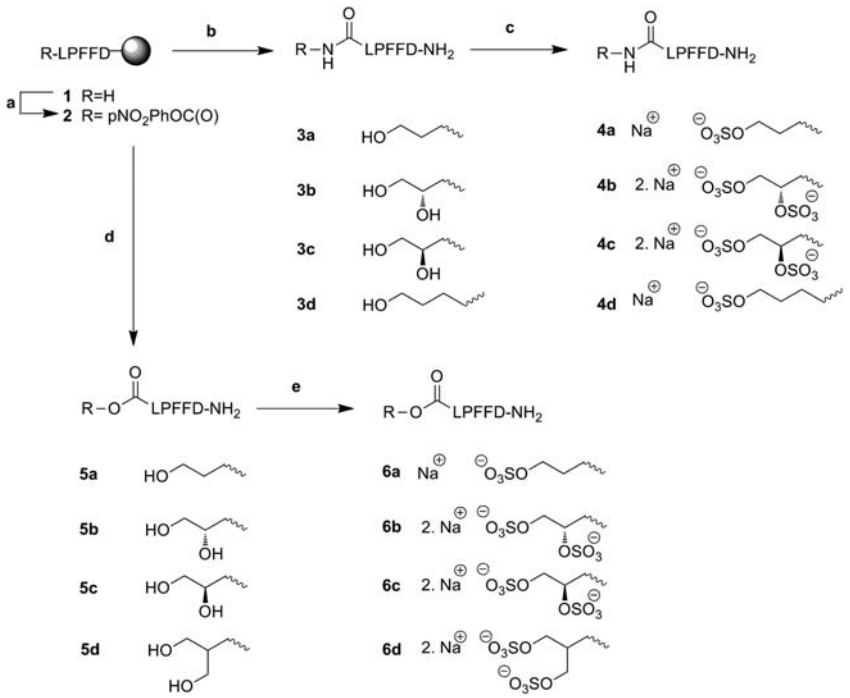

Fig. 1 Synthetic route towards hybrid ligands. Reaction conditions: (a) *p*-nitrophenylchloroformate and DIPEA in dichloromethane, r.t., 1 h; (b) HO-X-NH_2, *N*,*N*-diisopropylethylamine in DMF, r.t., 18 h; TFA/H_2O/1,2-ethanedithiol/triisopropylsilane (92.5/2.5/2.5/2.5), r.t., 2 h (**3a–3d**); (d) HO-X-OH, 1,8-diazabicycloundec-7-ene (DBU) in DMF, r.t., 18 h; TFA/H_2O/1,2-ethanedithiol/triisopropylsilane (92.5/2.5/2.5/2.5), r.t., 2 h (**5a–5c**) or 2-hydroxymethyl-1,3-propanediol, DBU in DMF, r.t., 18 h; dichloromethane/TFA/acetic acid (3/1/1), r.t., 1 h (**5d**); (c,e) sulfur trioxide-trimethylamine in DMF, 50 °C, 18 h; sat. aq. $NaHCO_3$, r.t.,18 h (**4a–4d** and **6a–6d**).

(Fig. 2B). Incubation of the compounds alone resulted in cell death comparable to control levels. Thus, N-terminal chain extension of peptide LPFFD with an aliphatic alcohol or sulfonylated alcohols, *via* urea or carbamate functionality, consequently leads to derivatives with reduced activity with respect to Ac-LPFFD-NH_2 (**7**) itself.

To find a possible explanation for these results, a molecular modeling approach was applied to the hybrid ligands (**3**,**4**,**5a–5c** and **6a–6c**) and the NMR structure deposited in RCSB Protein Data Bank under the ID number 2BEG[28] as a model for the $A\beta_{42}$ fibrils (see supporting materials). In earlier studies the binding site of LPFFD on $A\beta_{42}$ was determined[23,29] and we used this binding site to align our

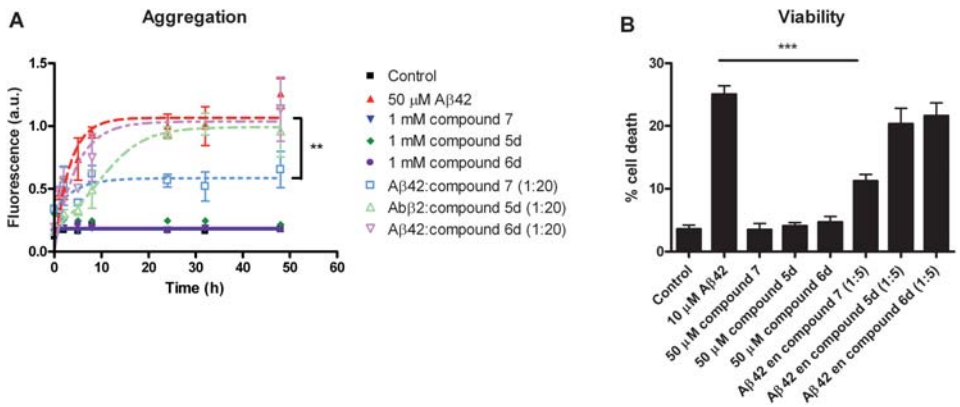

Fig. 2 Analysis of the effect of Ac-LPFFD-NH_2 (**7**), $C_5H_{10}O_4$-LPFFD-OH (**5d**) and $C_5H_8Na_2O_{10}S_2$-LPFFD-OH (**6d**) on Aβ-induced cytotoxicity towards human brain pericytes (HBPs). Effects on aggregation of 50 μM $A\beta_{42}$ for 48 h (A) and cell death in HBP cultures after incubation with 10 μM $A\beta_{42}$ for 6 days (B) is shown. The concentration of hybrid ligand is shown as the molar ratio of $A\beta_{42}$: hybrid ligand. Co-incubation of $A\beta_{42}$ with Ac-LPFFD-NH_2 (**7**) in a ratio of 1 : 20 results in 66% decrease in fluorescent signal ($p < 0.01$; A). However, co-incubation of $A\beta_{42}$ with $C_5H_{10}O_4$-LPFFD-OH or $C_5H_8Na_2O_{10}S_2$-LPFFD-OH in a ratio of 1 : 20 resulted in a fluorescent signal comparable to the signal of $A\beta_{42}$ alone (A). Incubation of buffer alone or the compounds themselves did not result in a fluorescent signal. In addition, a significant difference was observed in cell death between HBPs treated with 10 μM $A\beta_{42}$ alone (25% cell death) or with Ac-LPFFD-NH_2 (**7**; 11.3% cell death; $p < 0.001$; B) in a ratio of 1 : 5 for 6 days. However, no significant effect was observed when co-incubating $A\beta_{42}$ with $C_5H_{10}O_4$-LPFFD-OH *or* $C_5H_8Na_2O_{10}S_2$-LPFFD-OH in a ratio of 1 : 5 for 6 days compared to incubation with $A\beta_{42}$ alone (B). Incubation of the compounds alone resulted in a cell death comparable to control levels.

hybrid ligands to the $A\beta_{42}$ protein. As can be seen in Fig. S1, a nice β-pleated sheet was observed when aligning several $A\beta_{42}$ proteins. According to the obtained model, it was rationalized that a spacer of three carbon atoms should be sufficient to target His13 and/or His14 in the $A\beta_{42}$ protein with a sulfate group of the hybrid ligand, Fig. S1. Based on this insight, our synthetic compounds could simultaneously target the binding domains of GAGs (binding to Aβ amino acids 13–16) and of pentapeptides (binding to Aβ amino acids 16–22).

After alignment, molecular dynamics was performed with the assumption that the phenylalanine (amino acid 3) of the hybrid ligand adopts the same orientation as the phenylalanine (amino acid 18) of the $A\beta_{42}$ protein. Thus, from this initial conformation for molecular dynamics simulations in water, the energetically most favorable "docking" conformation was sought. It should be mentioned that during these calculations the starting configuration will not be the most sampled configuration if it is not energetically favorable, certainly if the simulation time is long enough. From these calculations it became apparent that, although both phenylalanines of the hybrid ligand adopt the same orientation as the phenylalanines of the $A\beta_{42}$ protein, it is difficult to target the histidines in the $A\beta_{42}$ protein with a non-peptide side-chain in the hybrid ligand. Rather, the side-chain bends away such that the sulfate groups interact with Lys15 of the $A\beta_{42}$ protein backbone, rather than with His13 or His14 (Fig. S2). Interestingly, during further molecular modeling simulations it became apparent that the hybrid ligands could be fully incorporated into the β-sheet structure of aggregated Aβ (Fig. S3), explaining why our hybrid ligands exerted no effect on Aβ aggregation and toxicity.

It is thought that BSB peptides, such as Ac-LPFFD-NH_2 (**7**), interfere with electrostatic interactions during aggregation or destabilize the Aβ-fibril internal hydrogen bond network, necessary to maintain the β-sheet structure, by forming strong hydrogen bonds with the Aβ subunits.[30] During the calculations we were solely focused at the optimization of the affinity of the sulfonylated hybrid ligands to the Aβ protein. The disadvantage of these compounds, however, is that because of this high quality binding, it is very likely that we have designed compounds that incorporate into the β-sheet structure of $A\beta_{42}$ aggregates, rather than inhibiting aggregation of the protein, due to restoring the Aβ fibril internal hydrogen bond network (Fig. S3). Thus, to be an effective BSB ligand, hybrid ligands should not only have a higher binding affinity to the $A\beta_{42}$ protein, but should also disrupt the β-sheet structure. Therefore, to optimize the ligands, a "bulky" sidechain/group could be introduced to optimize our hybrid ligands, thereby preventing its incorporation into the β-sheet structure of $A\beta_{42}$. An alternative strategy involves changing the amino acid sequence by replacing, for instance, one of the phenylalanines by a polar amino acid and/or reducing hydrogen bond interactions of the hybrid ligand with a next Aβ protein.

In conclusion, a hybrid ligand with improved characteristics with respect to inhibition of Aβ fibrillization and Aβ-mediated cytotoxicity compared to Ac-LPFFD-NH_2 (**7**)[10,14] was not found. However, as our molecular modeling experiments suggest, the designed hybrid ligands likely have a higher binding affinity for Aβ, but cannot avoid further aggregation of the Aβ protein. Changing the amino acid sequence by replacing, for instance, one of the phenylalanines by a polar amino acid and/or reducing hydrogen bond interactions of the hybrid ligand with a next Aβ protein could be a variable for optimization of the hybrid ligands in future studies to validate the hypothesis that hybrid ligands targeting Aβ fibrillization by a combination of peptide-peptide and sulfate-peptide interactions have enhanced properties.

Acknowledgements

We thank Dr R. Koopmans, Dr J. H. M Cox-Claessens and Dr G. Woestenburg (Psychogeriatric Centers "Joachim en Anna" and "Margriet", Nijmegen, The Netherlands) for their cooperation in the rapid autopsy protocol. We would also like to thank Peter van Galen and Hans Adams for their technical advice.

This study was supported by grants from the Internationale Stichting Alzheimer Onderzoek (ISAO, no. 03517 and 07510), the Netherlands Organisation for Scientific Research (NWO/ZonMW, Vidi program, no. 917.46.331) and the Hersenstichting Nederland (no. 14F06.18).

Reference list

1 D. Champagne, D. Pearson, D. Dea, J. Rochford and J. Poirier, *Neuroscience*, 2003, **121**, 99.
2 M. Citron, *Nat. Rev. Drug Discovery*, 2010, **9**, 387.
3 D. J. Selkoe, *Neuron*, 1991, **6**, 487.
4 A. A. Rensink, M. M. Verbeek, I. Otte-Holler, H. T. ten Donkelaar, R. M. de Waal and B. Kremer, *Brain Res.*, 2002, **952**, 111.
5 M. M. Verbeek, R. M. de Waal, J. J. Schipper and W. E. Van Nostrand, *J. Neurochem.*, 1997, **68**, 1135.
6 C. J. Pike, D. Burdick, A. J. Walencewicz, C. G. Glabe and C. W. Cotman, *J. Neurosci.*, 1993, **13**, 1676.
7 B. Brera, A. Serrano and M. L. de Ceballos, *Neurobiol. Dis.*, 2000, **7**, 395.
8 L. O. Tjernberg, J. Naslund, F. Lindqvist, J. Johansson, A. R. Karlstrom, J. Thyberg, L. Terenius and C. Nordstedt, *J. Biol. Chem.*, 1996, **271**, 8545.
9 C. Soto, M. S. Kindy, M. Baumann and B. Frangione, *Biochem. Biophys. Res. Commun.*, 1996, **226**, 672.
10 C. Soto, E. M. Sigurdsson, L. Morelli, R. A. Kumar, E. M. Castaño and B. Frangione, beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis. Implications for Alzheimer's therapy , *Nat. Med.*, 1998, **4**(7), 822–826.
11 M. A. Findeis, *Biochim.Biophys.Acta*, 2000, **1502**, 76.
12 C. Adessi, M. J. Frossard, C. Boissard, S. Fraga, S. Bieler, T. Ruckle, F. Vilbois, S. M. Robinson, M. Mutter, W. A. Banks and C. Soto, *J. Biol. Chem.*, 2003, **278**, 13905.
13 M. A. Chacon, M. I. Barria, C. Soto and N. C. Inestrosa, *Mol. Psychiatry*, 2004, **9**, 953.
14 B. Permanne, C. Adessi, G. P. Saborio, S. Fraga, M. J. Frossard, D. J. Van, I. Dewachter, W. A. Banks, L. F. Van and C. Soto, *FASEB J.*, 2002, **16**, 860.
15 C. Soto, M. S. Kindy, M. Baumann and B. Frangione, *Biochem. Biophys. Res. Commun.*, 1996, **226**, 672.
16 S. Hanessian, P. P. Lu, J. Y. Sanceau, P. Chemla, K. Gohda, R. Fonne-Pfister, L. Prade and S. W. Cowan-Jacob, *Angew. Chem., Int. Ed.*, 1999, **38**, 3159.
17 M. F. Gordeev, C. Hackbarth, M. R. Barbachyn, L. S. Banitt, J. R. Gage, G. W. Luehr, M. Gomez, J. Trias, S. E. Morin, G. E. Zurenko, C. N. Parker, J. M. Evans, R. J. White and D. V. Patel, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 4213.
18 C. Lerner, B. Masjost, A. Ruf, V. Gramlich, R. Jakob-Roetne, G. Zurcher, E. Borroni and F. Diederich, *Org. Biomol. Chem.*, 2003, **1**, 42.
19 R. Kisilevsky, L. J. Lemieux, P. E. Fraser, X. Kong, P. G. Hultin and W. A. Szarek, *Nat. Med.*, 1995, **1**, 143.
20 J. McLaurin and P. E. Fraser, *Eur. J. Biochem.*, 2000, **267**, 6353.
21 S. J. Pollack, I. I. Sadler, S. R. Hawtin, V. J. Tailor and M. S. Shearman, *Neurosci. Lett.*, 1995, **184**, 113.
22 C. Soto, M. S. Kindy, M. Baumann and B. Frangione, *Biochem. Biophys. Res. Commun.*, 1996, **226**, 672.
23 C. Hetenyi, T. Kortvelyesi and B. Penke, *Bioorg. Med. Chem.*, 2002, **10**, 1587.

24 H. Levine, III, *Protein Sci.*, 1993, **2**, 404.
25 M. M. Verbeek, I. Otte-Holler, P. Wesseling, D. J. Ruiter and R. M. de Waal, *Am. J. Pathol.*, 1994, **144**, 372.
26 M. M. Verbeek, J. R. Westphal, D. J. Ruiter and R. M. de Waal, *J. Immunol.*, 1995, **154**, 5876.
27 L. W. Hung, G. D. Ciccotosto, E. Giannakis, D. J. Tew, K. Perez, C. L. Masters, R. Cappai, J. D. Wade and K. J. Barnham, *J. Neurosci.*, 2008, **28**, 11950.
28 T. Lührs, C. Ritter, M. Adrian, D. Riek-Loher, B. Bohrmann, H. Döbeli, D. Schubert and R. Riek, 3D structure of Alzheimer's amyloid-beta(1–42) fibrils, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**(48), 17342–17347.
29 Z. Chen, G. Krause and B. Reif, *J. Mol. Biol.*, 2005, **354**, 760.
30 D. Chen, Z. S. Martin, C. Soto and C. H. Schein, *Bioorg. Med. Chem.*, 2009, **17**, 5189.

