Inhibition of fibril formation in β -amyloid peptide by a novel series of benzofurans

David R. HOWLETT^{*1}, Amanda E. PERRY^{*}, Fiona GODFREY^{*}, Jane E. SWATTON^{*}, Kevin H. JENNINGS^{*}, Claus SPITZFADEN^{*}, Harry WADSWORTH[†], Stephen J. WOOD^{*2} and Roger E. MARKWELL[†]

^{*}Department of Neuroscience, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park (North), Third Avenue, Harlow, Essex CM19 5AW, U.K., and

[†]Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park (North), Third Avenue, Harlow, Essex CM19 5AW, U.K.

A series of benzofuran derivatives have been identified as inhibitors of fibril formation in the β -amyloid peptide. The activity of these compounds has been assessed by a novel fibril-formation-specific immunoassay and for their effects on the production of a biologically active fibril product. The inhibition afforded by the compounds seems to be associated with their binding to β -amyloid, as identified by scintillation proximity binding assay. Binding assays and NMR studies also indicate that the inhibition is associated with self-aggregation of the compounds. There is a close correlation between the activity of

the benzofurans as inhibitors of fibril formation and their ability to bind to β -amyloid. Non-benzofuran inhibitors of the fibril formation process do not seem to bind to the same site on the β -amyloid molecule as the benzofurans. Thus a specific recognition site might exist for benzofurans on β -amyloid, binding to which seems to interfere with the ability of the peptide to form fibrils.

Key words: Alzheimer's disease, Congo Red, fibril formation inhibitor, immunoassay, scintillation proximity assay.

INTRODUCTION

The progressive deposition of β -amyloid (A β) in Alzheimer's disease is generally considered to be fundamental to the development of neurodegenerative pathology. Cell toxicity associated with A β provides an explanation for the neuronal cell loss found in Alzheimer's disease [1,2]. In particular, the neurotoxic effects in cell culture systems are dependent on the fibrillar state of the peptide [3,4] and on the degree and rate of fibril formation [5]. Many laboratories have demonstrated that aggregated or synthetic A β peptide in fibril form can promote neurodegeneration in cell culture systems (reviewed in [6]). Preventing the neurodegeneration by inhibiting the formation of toxic forms of A β is therefore a possible therapeutic approach to Alzheimer's disease.

A number of inhibitors of A β fibril formation have been described that prevent the formation of a biologically active species of peptide. We have previously demonstrated that β -cyclodextrin [7] and the haem-containing compounds haemin and haematin [8] inhibit fibril formation and hence prevent the generation of a biologically active form of A β . Other inhibitors include the rifamycin series [9] and sulphonated dyes [10]. There have been no reports on the effects of any of these compounds on A β deposition *in vivo*, although systemic amyloid formation has been attenuated by sulphonates [11] and 4'-iodo-4'-deoxydoxorubicin [12]. Short peptide fragments, based primarily on the central hydrophobic region of A β , have been proposed as being capable of preventing β -sheet formation [13,14]. A recent report has claimed that peptides of this type inhibit fibrillogenesis in a rat brain model of amyloidosis [15].

We describe here a series of benzofuran analogues identified as inhibitors of A β fibril formation and show the effectiveness of these compounds in preventing the formation of a fibrillar A β form by a process that seems to involve the binding of benzofuran to peptide.

MATERIALS AND METHODS

Materials

Suppliers of materials were as follows: A β 1–40 (lot nos. ZK600 and ZM605), Bachem (U.K.) Ltd.; ¹²⁵I-A β 1–40, DuPont–NEN; anti-mouse scintillation proximity assay (SPA) reagent, Amersham; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), rolitetracycline and benzoquinone, Sigma; daunomycin, Apin; antibody 6E10 (biotinylated and non-biotinylated), Senetek. All benzofurans and hexadecylmethylpiperadinium were obtained from the SmithKline Beecham compound collection. Monoclonal antibodies 2F12 and 1E8 were raised against the 1–16 and 13–27 fragments of A β 1–40 respectively [16]. Monoclonal antibody WO2 [17] was a gift from Dr. Tobias Hartmann (University of Heidelberg, Heidelberg, Germany) and was also raised against the 1–16 sequence of A β 1–40.

A β 1–40 was dissolved in 0.1% (v/v) acetic acid at 2 mg/ml (0.46 mM) immediately before use. Benzofurans (all as hydrochloride salts) were dissolved in DMSO at 10 mg/ml.

Congo Red screening assay

Inhibitory activity was determined exactly as described previously [18]. In brief, peptide plus inhibitor were incubated for 2 h at 25 °C in 50 mM Mes buffer, pH 5.8. After the addition of Congo Red solution [25 μ M Congo Red in 0.1 mM potassium phosphate/150 mM NaCl (pH 7.4)], the amount of Congo Red bound to the A β peptide was taken as proportional to the degree of fibril formation.

Immunoassay

Peptide and benzofurans were diluted in PBS, pH 7.4, containing 0.02% (w/v) Tween 20 to prevent binding of peptide to microtitre plates. Routinely, peptide at 11.6 μ M was incubated at 37 °C for

Abbreviations used: A β , β -amyloid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SKF-74652, [5-chloro-2-(4-methoxyphenyl)-3-benzofuran-1-yl]-4-[3-(diethylamino)propoxy]phenylmethanone hydrochloride; SPA, scintillation proximity assay.

¹ To whom correspondence should be addressed (e-mail David.Howlett-1@sbphrd.com).

² Present address: Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1789, U.S.A.

Figure 1 Detection of time-dependent A β fibril formation by immunoassay

Peptide was assayed fresh (vertical hatching), after 24 h of incubation (cross-hatching) or after 48 h of incubation (open bars). The immunoassay format (x-axis) comprised the capture of 2F12, 6E10 or WO2 and the detection of biotinylated 2F12 or 6E10. See the Materials and methods section for details. Results are means $\pm$ S.E.M. for three experiments.

the indicated durations in the presence or absence of competing compound. Incubations were performed in Linbro Titertek EIA II 96-well microtitre plates (ICN) covered with an acetate plate sealer (Dynatech Labs, catalogue no. 001-010 3501). Plates were not shaken. Peptide was then captured on microtitre plates (Nunc Maxisorb) precoated with the monoclonal antibodies 2F12 (2.7 μ g/ml), WO2 (2.7 μ g/ml) or 6E10 (1 μ g/ml). Detection was by means of biotinylated versions of the 2F12 (0.81 μ g/ml) and 6E10 (1 μ g/ml) monoclonal antibodies and binding of streptavidin-europium (Wallac, Milton Keynes, Bucks., U.K.) permitting quantification by DELFIA (delayed enhanced lanthanide fluorescence immunoassay; Wallac).

MTT assay

Peptide plus inhibitor incubations were as described above for the immunoassay. To assess cell viability, IMR32 human neuroblastoma cells (ECACC, Porton Down, Salisbury, Wilts., U.K.) were plated at 6×10^4 cells/cm² in a 96-well microtitre plate in Dulbecco's modified Eagle's medium/F12 growth medium containing 10% (v/v) foetal calf serum (Gibco). After 2 h of attachment, incubated test solutions (containing A β or benzofuran or both) were added at concentrations of 0.1–200 pmol/ml A β . After a further 24 h, MTT was added to a final concentration of 1 mg/ml and the cells were incubated for an additional 4 h. The reduction of MTT to a formazan product was assessed from the A_{490} after solubilization in DMSO [19].

Electron microscopy

Aliquots of peptide/inhibitor incubates were stained with 1% (w/v) uranyl acetate before examination in a Hitachi H7100 transmission electron microscope.

NMR

¹H-NMR spectra were acquired on a Varian Unity 300 MHz spectrometer, equipped with a 5 mm magic-angle-spinning probe head optimized for use with liquid samples. Microtubes with a 40 μ l sample volume were used. The benzofuran compounds

were added to aqueous amyloid solutions (concentration 150 mM in ²H₂O, pH 7, 22 °C) as concentrated solutions in DMSO, such that the final overall DMSO concentration in the sample did not exceed 5% (v/v).

SPA

[5-Chloro-2-(4-methoxyphenyl)-3-benzofuranyl]-[4-[3-(diethylamino)propoxy]phenyl]methanone hydrochloride (SKF-74652) was radiolabelled to 48.7 Ci/mmol by ³H exchange and was purified on a silica-gel Sep-Pak column (Waters) (Dr. R. Heys, SmithKline Beecham Pharmaceuticals, Upper Merion, PA, U.S.A.). A β 1–40 (11.6 μ M) was incubated for 18 h at room temperature on an orbital shaker in the presence of [³H]SKF-74652, 2F12 monoclonal antibody (5.4 μ g/ml) and anti-mouse SPA reagent. The association of [³H]ligand and A β was demonstrated by the formation of a complex between peptide, monoclonal antibody and SPA reagent, bringing the radioactive moiety and SPA reagent into proximity.

Binding of ¹²⁵I-A β 1–40

To assess whether compounds interfered with the deposition of A β , strip tubes (MJ Research, Watertown, MA, U.S.A.) were coated with a fibrillar amyloid substrate ('synthaloid'), exactly as described [20]. In brief, A β 1–40 in fibril form was suspended in a gelatin solution and then dried on the tube surfaces at 56 °C. The ability of compounds to interfere with the binding of [¹²⁵I]-A β 1–40 (NEN-DuPont) to synthaloid was taken as a reflection of their ability to regulate A β deposition.

RESULTS

Immunoassay

When A β 1–40 was incubated for 24 or 48 h, an increase in the fluorescence signal was observed, in comparison with that seen

Figure 2 Structures of benzofuran derivatives used

Table 1 Inhibition of $A\beta$ fibril formation assessed by immunoassay

$A\beta$ 1–40 (11.6 μ M) was incubated for 18 h in the presence or absence of competing compound. Fibrillar product was determined by immunoassay, as described in the Materials and methods section. Results are means $\pm$ S.E.M. for three experiments, unless indicated otherwise.

Compound	IC ₅₀ (μ M)
SKF-74652	28 $\pm$ 8
SB-240696	45 $\pm$ 16
SB-241070	45 $\pm$ 14
SKF-64346	56 $\pm$ 12
SKF-63210	80 $\pm$ 23
SKF-73735	85 (n = 2)
SKF-63058	> 1000
SKF-73033	> 1000
SKF-81733	> 1000

with freshly dissolved peptide. This increase was apparent when the capture antibody was 2F12, 6E10 or WO2 and the detection antibody was biotinylated 2F12 or 6E10 (Figure 1). With 1E8 antibody for capture and biotinylated 2F12 or 6E10 for detection, no such increase was noted and there was a tendency for the signal to decline with time.

Screening

The benzofuran series compounds, such as SKF-74652 and SKF-64346 (Figure 2), were originally identified as inhibitors using the Congo Red binding assay on the basis of the ability of the compounds, at 100 μ g/ml (23 μ M) to inhibit the binding of Congo Red to 400 μ g/ml (92 μ M) $A\beta$ 1–40. This assay was run

Figure 3 Effects of SKF-74652 on $A\beta$ 1–40 fibril-formation-dependent MTT reduction in IMR32 cells

$A\beta$ 1–40 (11.6 μ M) was converted into fibrils in the presence of SKF-74652 at 0 μ M ($\diamond$), 30 μ M ($\square$) or 100 μ M ($\triangle$) before cells were challenged as described in the Materials and methods section. Results are means $\pm$ S.E.M. for three separate experiments of triplicate determinations.

at pH 5.8, close to the pI for $A\beta$, to provide a rapid throughput assay system, with maximal Congo Red binding being achieved after 2 h incubation of the $A\beta$ 1–40. To provide a more physiological medium, subsequent incubations were undertaken at pH 7.4 with 18 h of incubation at 37 $^{\circ}$ C. These and related benzofurans with substitutions at the 2- and 3- positions (Figure 2) were assessed for activity in the fibril formation-specific immunoassay. Benzofurans with basic side chains generally had IC₅₀ values between 28 and 85 μ M, the exception being SKF-73033,

Figure 4 Electron micrographs of A β in fibril form in the presence or absence of the benzofuran SB-241070
(a) After 5 days of incubation of A β (11.6 μ M) alone; (b) after 5 days of incubation of A β in the presence of SB-241070 (200 μ M). Scale bars, 250 nm.

which was inactive at 1 mM (Table 1). This profile of activity was confirmed in the cell-based assay. In the absence of competing benzofuran, A β 1–40 in fibril form inhibited the MTT reduction with an EC₅₀ of 5 $\pm$ 1 nM (given as mean $\pm$ S.E.M.), with a maximal 40% reduction being observed at peptide concentrations of 50 nM or more. SKF-74652 (Figure 3) and SKF-64346, at 30 and 100 μ M, produced a shift to the right in the A β 1–40 concentration–response curve such that effects at less than 100 nM A β 1–40 were totally prevented. Again, SKF-73033 was without apparent activity (results not shown). The other active benzofurans, tested at 100 μ M, produced a marked inhibition of the responses to 10 and 100 nM A β 1–40 but had no direct effect on MTT reduction (results not shown). The above inhibitory effects were accompanied by a change in the fibrillar appearance of the A β . Thus, electron microscopic images of A β in fibril form shows numerous long straight/curving fibrils of

Figure 5 Effects of increasing concentrations of [3 H]SKF-74652 on binding to A β 1–40

Binding of [3 H]SKF-74652 to A β 1–40 was assessed by SPA, as described in the Materials and methods section. Results are from a representative experiment that was repeated three times. umole, μ mole.

Table 2 Inhibition of binding of [3 H]SKF-74652 to A β 1–40 as determined by SPA

A β 1–40 (11.6 μ M) was incubated for 18 h with 2F12 monoclonal antibody and SPA reagent in the presence or absence of benzofuran compound, as described in the Materials and methods section. Results are means $\pm$ S.E.M. for three to seven experiments. Abbreviation: n.d., not determined.

Compound	IC ₅₀ (μ M)
SKF-74652	11 $\pm$ 4
SB-240696	18 $\pm$ 2
SB-241070	13 $\pm$ 3
SKF-64346	16 $\pm$ 5
SKF-63210	n.d.*
SKF-73735	n.d.†
SKF-73033	> 200

* 30% inhibition at 15 μ M of added compound.

† 40% inhibition at 20 μ M of added compound.

Table 3 Comparison between inhibition of binding (by SPA) and inhibition of fibril formation (by immunoassay) for non-benzofurans

A β 1–40 (11.6 μ M) was incubated for 18 h in the presence or absence of competing compound. Fibrillar product was determined by immunoassay. To assess binding, the incubation included 2F12 monoclonal antibody and SPA reagent. Details of both assays are described in the Materials and methods section. Results are means $\pm$ S.E.M. for a minimum of three determinations. Abbreviation: n.d., not determined.

Compound	IC ₅₀ , SPA (μ M)	IC ₅₀ , fibril formation (μ M)
Hexadecylmethyl-piperadinium	18 $\pm$ 6	150 $\pm$ 44
Daunomycin	26 $\pm$ 12	30 $\pm$ 11
Rolitetraacycline	n.d.*	59 $\pm$ 8
Benzoquinone	> 1000	47 $\pm$ 21

* 10% inhibition at 20 μ M of added compound.

length more than 1 μ m, whereas in the presence of benzofuran inhibitors such as SB-241070 much shorter fibrils were apparent (Figure 4).

Figure 6 ^1H -NMR spectra of SKF-74652 in DMSO-d_6 (upper panel) and $^2\text{H}_2\text{O}$ (lower panel)

The aromatic signals, which are characteristic for the compound, are at 7–8.3 p.p.m.. The right-hand side in each spectrum (0–4 p.p.m.) contains signals from residual, undeuterated solvent and (in the lower panel) trimethylsilylpropionylsulphonate, which was used as a chemical shift reference.

Interactions between A β and benzofurans

It was not possible to determine the binding affinity of [^3H]SKF-74652 for A β 1–40 because the binding increased more or less linearly with increasing concentration of ligand in the presence or absence of competing benzofuran (Figure 5). Although competition was observed between tritiated and unlabelled SKF-74652, in a typical experiment the total binding of SKF-74652 increased exponentially from 0.16 nmol of SKF-74652/ μmol of A β 1–40 at 0.031 μM free SKF-74652 to 770 nmol of SKF-74652/ μmol of A β 1–40 at 203 μM free SKF-74652. Binding of [^3H]SKF-74652 was observed with freshly dissolved A β converted into fibrils (by incubation overnight), but not with peptide that had been treated with the α -helix-promoting solvent hexafluoro-propan-2-ol. The binding of [^3H]SKF-74652 to A β 1–40 was inhibited by compounds of the benzofuran series with IC_{50} values matching that obtained by immunoassay (Table 2). Other inhibitors of A β fibril formation did not inhibit the binding with potencies that could be predicted from their activities in the immunoassay (Table 3).

Figure 7 Effects of fibril formation inhibitors on binding of ^{125}I -A β to synthaloid

Synthaloid coated plates were incubated with ^{125}I -A β in the presence of SKF-74652 ($\square$), SKF-64346 ($\diamond$), rifampicin ($\triangle$) or daunomycin ($\times$), as described in the Materials and methods section. Results are expressed as a percentage of the ^{125}I -A β binding occurring in the absence of fibril formation inhibitors, and are means $\pm$ S.E.M. for three experiments.

Attempts to demonstrate association between peptide and inhibitor by NMR spectroscopy indicated that soluble high-molecular-mass aggregates (more than 20 kDa) of SKF-74652 (and SB-241070) occurred at high concentrations (150 μM) of benzofuran in aqueous solution at pH 7.0. Although SKF-74652, SB-241070 and A β 1–40 were soluble under these conditions, ^1H -NMR spectra exhibited severe line broadening, resulting in a complete loss of the specific NMR signals. This is illustrated for SKF-74652 by the loss of the NMR signal in the aromatic region (7.0–8.5 p.p.m.) of the $^2\text{H}_2\text{O}$ spectrum (Figure 6, lower panel) compared with a reference spectrum recorded in DMSO at the same concentration (Figure 6, upper panel). A similar loss of signal was observed for A β and for mixtures of A β and inhibitor.

The inhibitors examined produced a mixed profile of activity when assessed for their ability to interfere with the binding of ^{125}I -A β 1–40 to synthaloid. Some known inhibitors of fibril formation, such as rifampicin, also inhibited the binding of ^{125}I -A β 1–40 (Figure 7), whereas others, such as haemin and benzoquinone, were inactive (results not shown). In general, however, the benzofuran series of compounds consistently produced an increase in binding at concentrations of inhibitor greater than 100 μM (Figure 7). Aqueous benzofuran solutions more concentrated than 1 mM exhibited marked turbidity and did not show any effects on binding.

DISCUSSION

The assessment of A β anti-fibrillar or anti-aggregatory capacity of compounds has been approached in a number of ways, employing changes in either the physical properties of A β after polymerization, such as Congo Red binding [18], thioflavin T fluorescence [21] or binding studies [13] or in the biological effects of the fibrillar form in cell culture [5]. The use of a fibril-formation-specific immunoassay, as described above, permits high compound throughput and overcomes difficulties that can be encountered in other assays with coloured or fluorogenic compounds. The specificity is engendered by A β binding to the plate-bound capture antibody (2F12, WO2 or 6E10): only multimeric forms of the peptide bound to the plated antibody

provide exposed epitope for binding of the secondary biotinylated antibody. These three monoclonal antibodies, all raised against the 1–16 fragment of A β , seem to recognize a common epitope that is exposed in the peptide in fibril form. The precise nature of the A β species detected by this immunoassay configuration is not known at present but is under investigation. It is clear from the electron microscopy, however, that in conditions under which the production of an immunopositive product is inhibited, the long straight fibrils characteristic of fibrillar A β [5] are lost.

The biological activity of A β has been assessed by MTT assay in IMR32 human neuroblastoma cells. These cells are regarded as a suitable model for studying neurotoxic agents [22]. The neurotoxicity of A β has been described biochemically as an inhibition of MTT reduction or exocytosis [6,23] and an increase in the release of lactate dehydrogenase [24]. In the IMR32 cell system, A β in fibril form produced a maximum 40% decrease in MTT reduction at 50 nM or more. This supports the belief that MTT reduction does not measure cell death but is an index of the cell's metabolic integrity [25], which, as shown here, can be compromised at concentrations of A β as low as 1 nM, well below the A β concentrations that are usually necessary to produce cell death [1,13,14,24].

The appropriateness of the immunoassay system is demonstrated by the correlation between activity in that assay and the ability of compounds to prevent the formation of biologically active A β . This correlation and the observed effects of the inhibitors in the biophysical assays suggests that the biological effects of compound are on the MTT reduction dependent on fibrillar A β and not on the clearance or degradation of A β . Hence compounds such as SKF-74652 and SKF-64346 show comparable inhibitory activities in both assay systems, whereas SKF-73033 is inactive. The lack of activity of SKF-73033 is somewhat surprising considering the activity of the related benzofurans but an explanation might lie in the position of the bulky 3,5-diethyl-substituted phenyl ring in relation to the basic ethanolamine side chain. The lack of anti-fibrillar activity of benzofurans with no basic side chain (e.g. SKF-63058 and SKF-81733) highlights the importance of this moiety, which might be attached to either the 2- or the 3- position of the benzofuran ring. The inhibitory potencies observed for the active inhibitors, for which the immunoassay IC₅₀ values describe their ability to prevent fibril formation in 11 μ M A β 1–40, suggests that the peptide–inhibitor interaction is, at best, of 1:1 stoichiometry (and the apparent self-aggregation of the benzofuran compounds indicates that the ratio is less than 1:1). Hence, if the high-order kinetics observed for A β aggregation *in vitro* [26] (which requires the presence of micromolar peptide concentrations) pervade *in vivo*, then micromolar inhibitor concentrations might be required in plaque-susceptible brain areas to prevent the deposition of A β .

An understanding of the interaction between A β and inhibitor at molecular level is complicated by their aggregation properties under physiological conditions. The NMR characteristics showed that the species formed under these conditions had extremely long rotational correlation times, indicating that the compounds were present exclusively in the form of soluble high-molecular-mass aggregates (more than 40 kDa). This result was confirmed by dynamic light scattering (R. Jepras, unpublished work). This aggregation process occurred within the dead-time of the experiment (approx. 1 h) and was reversible on increasing the pH to 11. Hence the material apparently in fibril form observed by NMR was clearly unrelated to fibril formation but might be an important early stage of the fibril formation process. The NMR results were in agreement with the results of the Congo Red assay and immunoassay, in which freshly dissolved A β and non-incubated peptide had no effect on MTT reduction in IMR32

cells (results not shown). Similarly, the initial fibril formation process was not affected by the presence of inhibitor, suggesting that the compound did not interact with monomeric A β or dissociate the initial aggregate into stoichiometrically defined low-molecular-mass A β –inhibitor complexes. It therefore seems that the inhibitor interacts with an A β species farther down the fibril formation pathway. The suggestion that the benzofurans might not interact with monomer is further supported by the lack of binding of [³H]SKF-74652 to A β that had been treated with the α -helix-promoting solvent hexafluoropropan-2-ol, a treatment that has been reported to remove seed and leave an essentially monomeric preparation [27].

Comparable anti-fibrillar activity was observed with the paramagnetically tagged nitroxide-containing compounds SB-241070 and SB-240696. These compounds were synthesized in an attempt to use the distance-dependent NMR relaxation-enhancing properties of the paramagnetic centre to provide geometrical information about the binding site or sites of the fibril formation inhibitors. However, fibril formation in the peptide under the conditions of high concentration and neutral pH of the NMR experiments precluded the unambiguous interpretation of the results of titrations of peptide with these 'spin probes' in terms of binding geometry.

However, binding studies clearly showed an association between A β and the fibril formation inhibitor SKF-74652 and an ability of other benzofurans to compete for this binding with potencies similar to that observed in assays measuring the inhibition of fibril formation. The lack of consistent effect of non-benzofuran fibril-formation inhibitors to compete for this binding clearly suggests that multiple binding sites for inhibitors exist on the A β molecule. The increases in binding observed at high concentrations of inhibitors and the increase in binding of ¹²⁵I-A β 1–40 to the synthaloid template, together with the NMR results, suggest that the benzofurans are capable of self-aggregation and that A β –benzofuran aggregate complexes lacking biological activity might be formed during incubations with the benzofuran, forming the template for further binding. Hence these compounds could not be described as 'amyloid-deposition inhibitors' [28].

In summary, we have described a novel class of inhibitors of A β fibril formation that prevent the formation of multimeric species of A β that are biologically active in cell cultures. Proof of this concept will only be achieved by the demonstration of the effectiveness of compounds such as these in transgenic mouse models of Alzheimer's disease and ultimately in humans.

REFERENCES

- 1 Yankner, B. A., Dawes, L. R., Fisher, S., Villa-Komaroff, L., Oster-Granite, M. L. and Neve, R. L. (1989) *Science* **245**, 417–420
- 2 Yankner, B. A., Duffy, L. K. and Kirschner, D. A. (1990) *Science* **250**, 279–282
- 3 Pike, C. J., Walencewicz, A. J., Glabe, C. G. and Cotman, C. W. (1991) *Brain Res.* **563**, 311–314
- 4 May, P. C., Gitter, B. D., Waters, D. C., Simmons, L. K., Becker, G. W., Small, J. S. and Robison, P. M. (1992) *Neurobiol. Aging* **13**, 605–607
- 5 Howlett, D. R., Jennings, K. H., Lee, D. C., Clark, M. S. G., Brown, F., Wetzel, R., Wood, S. J., Camilleri, P. and Roberts, G. W. (1995) *Neurodegeneration* **4**, 23–32
- 6 Iversen, L. L., Mortishiresmith, R. J., Pollack, S. J. and Shearman, M. S. (1995) *Biochem. J.* **311**, 1–16
- 7 Camilleri, P., Haskins, N. J. and Howlett, D. R. (1994) *FEBS Lett.* **341**, 256–258
- 8 Howlett, D., Cutler, P., Heales, S. and Camilleri, P. (1997) *FEBS Lett.* **417**, 249–251
- 9 Tomiyama, T., Shoji, A., Kataoka, K., Suwa, Y., Asano, S., Kaneko, H. and Endo, N. (1996) *J. Biol. Chem.* **271**, 6839–6844
- 10 Pollack, S. J., Sadler, I. I., Hawtin, S. R., Taylor, V. J. and Shearman, M. S. (1995) *Neurosci. Lett.* **197**, 211–214
- 11 Kisilevsky, R., Lemieux, L. J., Fraser, P. E., Kong, X., Hultin, P. G. and Szarek, W. A. (1995) *Nat. Med. (Tokyo)* **1**, 143–148

- 12 Tagliavini, F., McArthur, R. A., Canciani, B., Giaccone, G., Porro, M., Bugiani, M., Lievens, P. M., Bugiani, O., Peri, E., Dall'Ara, P. et al. (1997) *Science* **276**, 1119–1122
- 13 Soto, C., Kindy, M. S., Baumann, M. and Frangione, B. (1996) *Biochem. Biophys. Res. Commun.* **226**, 672–680
- 14 Tjernberg, L. O., Lilliehook, C., Callaway, D. J., Naslund, J., Hahne, S., Thyberg, J., Terenius, L. and Nordstedt, C. (1997) *J. Biol. Chem.* **272**, 12601–12605
- 15 Soto, C., Sigurdsson, E. M., Morelli, L., Kumar, R. A., Castano, E. M. and Frangione, B. (1998) *Nat. Med. (Tokyo)* **4**, 822–826
- 16 Allsop, D., Christie, G., Gray, C., Holmes, S., Markwell, R., Owen, D., Smith, L., Wadsworth, H., Ward, R. V., Hartmann, T. et al. (1997) in *Alzheimer's Disease: Biology, Diagnosis and Therapeutics* (Iqbal, K., Winblad, B., Nishimura, T., Takeda, M. and Wisniewski, H. M., eds), pp. 717–727, John Wiley & Sons, New York
- 17 Ida, N., Hartmann, T., Pantel, J., Schroder, J., Zerfass, R., Forstl, H., Sandbrink, R., Masters, C. L. and Beyreuther, K. (1996) *J. Biol. Chem.* **271**, 22908–22914
- 18 Wood, S. J., Mackenzie, L., Maleeff, B., Hurle, M. R. and Wetzel, R. (1996) *J. Biol. Chem.* **271**, 4086–4092
- 19 Hansen, M. B., Nielsen, S. E. and Berg, K. (1989) *J. Immunol. Methods* **119**, 203–210
- 20 Esler, W. P., Stimson, E. R., Ghilardi, J. R., Felix, A. M., Lu, Y. A., Vinters, H. V., Mantyh, P. W. and Maggio, J. E. (1997) *Nat. Biotechnol.* **15**, 258–263
- 21 LeVine, H. (1993) *Protein Sci.* **2**, 404–410
- 22 Williams, S. P., O'Brien, S., Whitmore, K., Purcell, W. H., Cookson, M. R., Pentreath, V. W. and Atterwill, C. K. (1996) *In Vitro Toxicol. J. Mol. Cell. Toxicol.* **9**, 83–92
- 23 Liu, Y. B. and Schubert, D. (1997) *J. Neurochem.* **69**, 2285–2293
- 24 Behl, C., Davis, J. B., Lesley, R. and Schubert, D. (1994) *Cell* **77**, 817–827
- 25 Tan, S., Maher, P. and Schubert, D. (1997) *Brain Res.* **765**, 159–163
- 26 Jarrett, J. T. and Lansbury, Jr., P. T. (1993) *Cell* **73**, 1055–1058
- 27 Wood, S. J., Chan, W. and Wetzel, R. (1996) *Biochemistry* **35**, 12623–12628
- 28 Maggio, J. E. and Mantyh, P. W. (1996) *Brain Pathol.* **6**, 147–162

Received 13 January 1999/24 February 1999; accepted 5 March 1999