Identification of approved drugs that inhibit the binding of amyloid β oligomers to ephrin type-B receptor 2

Koichiro Suzuki1,2, Takahiro Aimi1, Tomoaki Ishihara1 and Tohru Mizushima3

1 Division of Drug Discovery and Development, Faculty of Pharmacy, Keio University, Minato-ku, Tokyo, Japan
2 Research Fellow of Japan Society for the Promotion of Science, Chiyoda-ku, Tokyo, Japan
3 LTT Bio-Pharma Co., Ltd, Minato-ku, Tokyo, Japan

Keywords
Alzheimer’s disease; amyloid β oligomer; ephrin type-B receptor 2

Correspondence
T. Ishihara, Division of Drug Discovery and Development, Faculty of Pharmacy, Keio University, 1-5-30, Shibakoen, Minato-ku, Tokyo 105-8512, Japan
Fax/Tel: +81 3 5400 2624
E-mail: ishihara-tm@pha.keio.ac.jp

(Received 16 January 2016, revised 26 February 2016, accepted 10 March 2016)
doi:10.1002/2211-5463.12056

Ephrin type-B receptor 2 (EphB2) is a member of the receptor tyrosine kinase family and plays an important role in learning and memory functions. In patients with Alzheimer’s disease (AD) and in mouse models of AD, a reduction in the hippocampal EphB2 level is observed. It was recently reported that normalization of the EphB2 level in the dentate gyrus rescues memory function in a mouse model of AD, suggesting that drugs that restore EphB2 levels may be beneficial in the treatment of AD. Amyloid β (Aβ) oligomers, which are believed to be key molecules involved in the pathogenesis of AD, induce EphB2 degradation through their direct binding to EphB2. Thus, compounds that inhibit the binding of Aβ oligomers to EphB2 may be beneficial. Here, we screened for such compounds from drugs already approved for clinical use in humans. Utilizing a cell-free screening assay, we determined that dihydroergotamine mesilate, bromocriptine mesilate, cepharanthine, and levonorgestrel inhibited the binding of Aβ oligomers to EphB2 but not to cellular prion protein, another endogenous receptor for Aβ oligomers. Additionally, these four compounds did not affect the binding between EphB2 and ephrinB2, an endogenous ligand for EphB2, suggesting that the compounds selectively inhibited the binding of Aβ oligomers to EphB2. This is the first identification of compounds that selectively inhibit the binding of Aβ oligomers to EphB2. These results suggest that these four compounds may be safe and effective drugs for treatment of AD.

Alzheimer’s disease (AD) is a progressive neurodegenerative disease and the most common cause of dementia. Because the world’s population is aging, the number of AD patients has been increasing; however, there are currently no disease-modifying treatments for AD. Thus, development of disease-modifying drugs is important.

One hallmark pathological feature of AD is senile plaques, which are composed of amyloid β peptide (Aβ). Monomeric Aβ easily self-assembles to form soluble oligomers, protofibrils, and fibrils. A correlation between the amount of soluble Aβ oligomers and cognitive impairment in AD patients has been reported [1]. In addition, soluble Aβ oligomers inhibit long-term potentiation (LTP) [2,3] which is involved in learning and memory functions [4]. Therefore, Aβ oligomers are believed to play an important role in the learning and memory dysfunction observed in patients with AD.

Abbreviations
ABTS, 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AD, Alzheimer’s disease; Aβ, amyloid β peptide; BRO, bromocriptine mesilate; BSA, bovine serum albumin; CEP, cepharanthine; DIH, dihydroergotamine mesilate; DMEM, Dulbecco’s modified Eagle medium; DMSO, dimethyl sulfoxide; EphB2, ephrin type-B receptor 2; HFIP, 1,1,1,3,3,3-hexafluoro-2 propanol; HRP, horseradish peroxidase; LCA, lithocholic acid; LEV, levonorgestrel; LTP, long-term potentiation; PBS, phosphate-buffered saline; PrPC, cellular prion protein; SD, standard deviation.
Inhibitors for the binding of Aβ to EphB2

Some endogenous receptors for Aβ oligomers, such as ephrin type-B receptor 2 (EphB2) and cellular prion protein (PrP C), appear to be involved in the inhibitory effects of Aβ oligomers on LTP [5–7]. EphB2, a member of the receptor tyrosine kinase family [8], is a receptor for ephrinB ligands (B1, B2, and B3) and ephrinA5 [9] and functions in synaptic plasticity and synapse formation [10–12]. Mice lacking EphB2 exhibit LTP impairment and memory dysfunction [11,12]. Reduced EphB2 levels are observed in postmortem hippocampal tissue from patients with AD [13] and in mouse models of AD [5,13,14]. Aβ oligomers directly bind to EphB2 and induce its internalization and proteasome-mediated degradation [5]. In addition, artificial expression of EphB2 in the dentate gyrus rescues LTP and memory function in a mouse model of AD [5]. These results suggest that inhibiting Aβ oligomer binding to EphB2 may be therapeutically beneficial in the treatment of AD.

The number of drugs reaching the marketplace each year is decreasing, mainly due to unexpected adverse effects being revealed at advanced stages of clinical trials. To overcome this problem, we took advantage of a recent strategy for drug discovery and development that focuses on examining drugs already approved for use in humans (i.e., drug repositioning). In this strategy, compounds with clinically beneficial pharmacological activity are screened from a library of approved drugs already in clinical use to be developed for new indications. One major advantage of this strategy is a decreased risk for unexpected adverse effects in humans because the safety of these drugs has already been well characterized in humans [15].

In the present study, from a library of approved drugs already in clinical use, we screened for compounds that inhibited the binding of Aβ oligomers to EphB2. We identified dihydroergotamine mesilate (DIH), bromocriptine mesilate (BRO), cepharanthine (CEP), and levonorgestrel (LEV). Although these compounds inhibited the binding of Aβ oligomers to EphB2, they did not inhibit the binding of an endogenous ligand, ephrinB2, to EphB2. This is the first time that compounds that selectively inhibited the binding of Aβ oligomers to EphB2 have been identified.

Materials and methods

Materials

Biotin-conjugated Aβ42 (biotin-Aβ) was obtained from AnaSpec Inc. (Fremont, CA, USA). EphB2 receptor ectodomains fused to the Fc portions of human IgG (EphB2-Fc) and biotinylated ephrinB2-Fc were purchased from R&D systems Inc. (Minneapolis, MN, USA). His-tagged human PrP C was obtained from Merck Co. (Darmstadt, Germany). Bovine serum albumin (BSA) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Co. (St. Louis, MI). Horseradish peroxidase-conjugated streptavidin (HRP-streptavidin) was obtained from GE Healthcare UK Ltd. (Little Chalfont, UK). The 2,2',2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD, USA). The antibodies against Aβ (6E10 and PrP C (6E10) and PrP C (ICSM35) were purchased from Covance Inc. (Emeryville, CA, USA) and D-Gen Co. (London, UK), respectively. Phenol red-free Dulbecco’s modified Eagle medium (DMEM) and 1,1,1,3,3,3-hexafluoro-2 propanol (HFIP) were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The library of drugs approved for clinical use was obtained from our laboratory stock [16].

Aβ monomer and oligomer preparation

Aβ monomers and oligomers were prepared as described previously [16]. Biotin-Aβ was dissolved in HFIP and incubated at room temperature for 1 h and then on ice for 10 min. The HFIP was evaporated and the peptides were dissolved in DMSO for a final concentration of 10 mM. The peptides were diluted in phenol red-free DMEM to give a final concentration of 100 μM and subjected to sonication (AIWA Co., Tokyo, Japan) for 10 min.

For oligomer preparation, the Aβ solution described above was incubated at 37 °C for 16 h and then centrifuged at 20 400 g for 15 min. The supernatant was frozen in liquid nitrogen and stored at –80 °C until use.

For monomer preparation, the sample was centrifuged and stored in a manner similar to that for oligomer preparation, without the incubation at 37 °C for 16 h.

Characterization of Aβ oligomers by immunoblotting

To characterize Aβ oligomers, the supernatant was mixed with equal amounts of sample buffer and boiled at 98 °C for 5 min. Samples were applied to polyacrylamide sodium dodecyl sulfate gels and subjected to electrophoresis, after which proteins were immunoblotted with 6E10.

Cell-free binding assay

The cell-free binding assay was performed as described previously [16]. To evaluate binding between Aβ and EphB2, EphB2-Fc was dissolved in phosphate-buffered saline (PBS) to a final concentration of 0.5 μg/mL and applied to a 96-well ELISA plate and left at 4 °C overnight. Each well was washed with PBS containing 0.05% Tween 80 (T-PBS) and blocked with 2% BSA at room temperature for 1 h. Then, each well was washed with T-PBS and incubated with both
biotin-Aβ (200 nM) and each tested drug in DMEM at room temperature for 1 h. Each well was then washed with T-PBS and incubated with HRP-streptavidin in PBS (1 : 2000 dilution) at room temperature for 30 min. Each well was washed with T-PBS and incubated with ABTS. Absorbance at 412 nm was measured using a plate reader (Infinite M1000; TECAN Group Ltd., Mannedorf, Switzerland). Biotin-Aβ binding to EphB2 was calculated as follows:

- **OD<sub>Blank</sub>**: absorbance of well without both EphB2 and each tested drug
- **OD<sub>Control</sub>**: absorbance of well with EphB2 but without any tested drug
- **OD<sub>Drug</sub>**: absorbance of well with both EphB2 and each tested drug

\[
A\beta \text{ binding}(\text{OD}_{412}) = \frac{\text{OD}_{\text{Drug}} - \text{OD}_{\text{Blank}}}{\text{OD}_{\text{Control}} - \text{OD}_{\text{Blank}}} \times 100
\]

As a quality parameter for this assay, **Z'-factor** [17] was calculated as follows:

\[
Z' = 1 - \frac{3 \times \sigma_{\text{Control}} + 3 \times \sigma_{\text{Blank}}}{|\mu_{\text{Control}} - \mu_{\text{Blank}}|}
\]

To evaluate binding between Aβ and PrP<sup>C</sup>, EphB2-Fc was replaced with His-tagged human PrP<sup>C</sup>.

To evaluate binding between ephrinB2 and EphB2, biotin-Aβ in DMEM was replaced with biotinylated ephrinB2-Fc in PBS (500 ng·mL<sup>−1</sup>).

**Chemical library and screening**

Each drug in the library was dissolved in water or DMSO, depending on the drug. The concentration of each drug in the library was 1 mM, 5 mM, 10 mM, 25 mM, 50 mM, 100 mM, 2 mg·mL<sup>−1</sup>, 10 mg·mL<sup>−1</sup>, or 100 mg·mL<sup>−1</sup>, depending on the drug. Each drug was diluted 1000 times for the screening (final concentration, 1 μM, 5 μM, 10 μM, 25 μM, 50 μM, 100 μM, 2 μg·mL<sup>−1</sup>, 10 μg·mL<sup>−1</sup>, or...
100 μg·mL⁻¹). We confirmed that 0.1% of water or DMSO did not affect this assay. Screening was performed in duplicate wells per each drug in the same reader plate.

**Statistical analysis**

All values are expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey’s test was used to evaluate differences among three or more groups. Differences were considered statistically significant at P values less than 0.05.

### Results and Discussion

**Screening of approved drugs for compounds that inhibit the binding of Aβ oligomers to EphB2**

To screen for inhibitors of Aβ oligomer binding to EphB2, we used a cell-free binding assay. In this assay, biotin-Aβ was applied to EphB2-coated wells, and after washing, the amount of bound biotin-Aβ was determined using HRP-streptavidin. Aβ oligomers were prepared by incubation at 37 °C for 16 h, and we confirmed the formation of Aβ oligomers by immunoblotting (Fig. 1A).

We first compared the ability of Aβ monomers and oligomers to bind EphB2. We found that Aβ oligomers bound to EphB2 more efficiently than did the monomers (Fig. 1B). This result was consistent with a previous report [5] and suggested that this assay was appropriate for screening compounds that inhibited the binding of Aβ oligomers to EphB2.

From a library of 840 drugs approved for clinical use, we screened for compounds that inhibited the binding of Aβ oligomers to EphB2. Each compound was applied to the cell-free binding assay simultaneously with Aβ oligomers, and the amount of bound biotin-Aβ was determined (Fig. 1C). As an assay quality parameter, we calculated Z'-factor to be 0.89.

![Image]

**Table 1. Inhibitory effect of each compound on the binding of Aβ oligomers to EphB2 or PrP C.**

| Compound                      | EphB2 Screening | Reproducibility | PrP C          |
|-------------------------------|-----------------|-----------------|----------------|
| Montelukast sodium            | 3.5             | 0.0             | 6.0 ± 0.4      |
| Pirarubicin hydrochloride     | 4.6             | 19.7 ± 1.8      | 33.1 ± 3.6     |
| Minocycline hydrochloride     | 8.8             | 3.9 ± 1.4       | 4.7 ± 2.1      |
| Tosufoxacin tosilate          | 12.2            | 11.2 ± 0.7      | 19.1 ± 2.6     |
| Diclazuril                    | 13.3            | 11.3 ± 1.7      | 20.8 ± 5.1     |
| Suramin sodium                | 14.7            | 16.4 ± 1.9      | 21.2 ± 0.6     |
| Mitelofosine                  | 16.0            | 50.1 ± 1.9      | 53.0 ± 4.4     |
| Cytochrome c                  | 16.4            | 27.1 ± 1.9      | 32.0 ± 2.0     |
| L-thyroxine                   | 28.1            | 53.8 ± 7.0      | 60.1 ± 3.7     |
| Abamectin                     | 29.8            | 40.1 ± 2.1      | 45.4 ± 5.7     |
| Nystatin                      | 35.6            | 33.2 ± 4.7      | 57.5 ± 0.8     |
| Lysozyme hydrochloride        | 40.8            | 48.7 ± 3.8      | 60.6 ± 2.0     |
| Bexarotene                    | 48.5            | 32.7 ± 1.7      | 46.2 ± 1.2     |
| Dihydroergotamine mesilate    | 48.6            | 62.7 ± 6.7      | 65.3 ± 5.6     |
| Retinoic acid                 | 48.9            | 39.5 ± 13.9     | 62.8 ± 2.3     |
| Mifepristone                  | 53.3            | 49.1 ± 8.8      | 55.7 ± 2.2     |
| Bromocriptine mesilate        | 53.9            | 57.9 ± 6.3      | 69.5 ± 5.8     |
| Toltrazuril                   | 58.6            | 55.8 ± 1.6      | 61.2 ± 8.7     |
| Cepharanthine                 | 60.2            | 50.1 ± 4.0      | 66.3 ± 7.4     |
| Indigocarmine                 | 62.2            | 55.9 ± 2.4      | 64.1 ± 8.5     |
| Levonorgestrel                | 62.6            | 64.1 ± 2.0      | 65.2 ± 4.2     |
| Oxytetracycline hydrochloride | 63.2            | 53.7 ± 3.2      | 65.7 ± 4.8     |

Among 52 compounds that inhibited more than 30% of the Aβ oligomer binding to EphB2 in the screening, 22 compounds that inhibited more than 30% of the Aβ oligomer binding to EphB2 in additional two independent experiments are listed. The effects of these 22 compounds on the binding of Aβ oligomers to EphB2 in the screening and in the additional two independent experiments (reproducibility) and on the binding of Aβ oligomers to PrP C were shown. The four compounds that inhibited less than 30% of the Aβ oligomer binding to PrP C are highlighted in gray. Values are mean (n = 2, “Screening” column) or mean ± SD (n = 3). The concentration of nystatin or retinoic acid was 50 μM in the screening or 100 μM in other experiments. The concentration of cytochrome c or lysozyme hydrochloride was 10 μg·mL⁻¹ in all experiments. The concentration of other all compounds was 100 μM in all experiments.
supporting the quality of this assay. Among these 840 compounds, 52 compounds inhibited more than 30% of the Aβ oligomer binding to EphB2. We examined the effect of these compounds on the binding in additional two independent experiments. We selected 22 compounds that inhibited more than 30% of the Aβ oligomer binding to EphB2 in both experiments (Table 1, red dots in Fig. 1C).

Through this screening procedure, we also found five compounds that enhanced the binding of Aβ oligomers to EphB2 to more than 200% (Table 2).

To select compounds that selectively inhibited the Aβ oligomer binding to EphB2, we next examined the effects of these 22 compounds on the binding of Aβ oligomers to PrP\(^{C}\). We found four compounds (DIH, BRO, CEP, and LEV; Fig. 2A) that inhibited less than 30% of the Aβ oligomer binding to PrP\(^{C}\) (Table 1). As shown in Fig. 2B, each of these four compounds inhibited the binding of Aβ oligomers to EphB2 in a concentration-dependent manner. Additionally, under

**Table 2.** Compounds that enhance the binding of Aβ oligomers to EphB2.

| Compound                  | Aβ binding (% of control) |
|---------------------------|---------------------------|
| Domiphen bromide          | 386.8                     |
| Dopamine hydrochloride    | 262.8                     |
| Pentamidine isethionate   | 238.6                     |
| Levodopa                  | 214.9                     |
| Fluvoxamine maleate       | 206.6                     |

Five compounds that increased the Aβ oligomer binding to EphB2 to more than 200% in the screening are listed. Values are mean (n = 2). The concentration of drugs was 100 \(\mu \text{M}\).

**Fig. 2.** Selective inhibition of the binding of Aβ oligomer to EphB2 by selected compounds. (A) Structures of the four selected compounds are shown. (B) The indicated concentration of each compound was added simultaneously with Aβ oligomers to EphB2-coated wells, and the amount of bound biotin-Aβ was determined. (C) Each compound (100 \(\mu \text{M}\) or ICSM35 (2 \(\mu \text{g}\cdot\text{mL}^{-1}\)) was added simultaneously with Aβ oligomer to PrP\(^{C}\)-coated wells, and the amount of bound biotin-Aβ was determined. Values represent the mean ± SD (n = 3). **P < 0.01. Basically similar results were obtained in another independent experiment.
Inhibitors for the binding of Aβ to EphB2

K. Suzuki et al.

conditions in which an antibody against PrP<sup>C</sup> (ICSM35) [7] inhibited the binding of Aβ oligomers to PrP<sup>C</sup>, none of these four compounds inhibited the binding (Fig. 2C). These results suggested that these four compounds selectively inhibited the binding of Aβ oligomers to EphB2.

Effects of selected compounds on the binding of ephrinB2 to EphB2

The results in Fig. 2 suggested that the four selected compounds interacted with EphB2 rather than with Aβ oligomers to inhibit their binding. Thus, it is possible that these compounds may inhibit the binding between EphB2 and its endogenous ligands, which may cause adverse clinical effects. Thus, we examined the effects of these four compounds on the binding of one of the endogenous ligands, ephrinB2, to EphB2. As shown in Fig. 3, none of these four compounds inhibited the binding of ephrinB2 to EphB2. We confirmed the inhibitory effect of lithocholic acid, which interferes with Eph–ephrin interactions [18], on the binding of ephrinB2 to EphB2 (Fig. 3).

Recently, some compounds were reported to inhibit the binding of Aβ to its receptors. For example, Deane et al. [19] identified FPS-ZM1 as an inhibitor for the binding of Aβ to the receptor for advanced glycation end products, and Risse et al. identified Chicago Sky Blue 6B as an inhibitor for the binding of Aβ to PrP<sup>C</sup> [20]. Furthermore, Fu et al. reported that rhynchophylline binds to EphA4 and stimulated LTP in the presence of Aβ oligomer [21].

As described above, Aβ oligomers directly bind to EphB2 and induce its degradation, resulting in LTP impairment and memory dysfunction [5]. Because artificial induction of EphB2 expression in the dentate gyrus rescues LTP and memory function in a mouse model of AD [5], induction of EphB2 expression is a potential drug target for treatment of AD. However, EphB2 is also involved in the development of cancer [22,23] and may stimulate cancer progression. Thus, inhibiting the binding between Aβ oligomers and EphB2 is a better target for developing drugs aimed at AD treatment. Here, we found 22 compounds with such activity. Among them, 18 compounds also inhibited the binding of Aβ oligomers to PrP<sup>C</sup>, whereas four compounds did not. Although we focused on the latter group for development of AD therapeutics, the former group is also interesting. In addition to EphB2, other receptors for Aβ oligomers, such as PrP<sup>C</sup> [6,7,24], leukocyte immunoglobulin-like receptor B2 [25], and IgG Fcγ receptor II-b [26], are involved in the inhibitory effect on LTP and in neurotoxicity.

Therefore, compounds that inhibit the binding of Aβ oligomers to various receptors may have beneficial effects for treatment of patients with AD. Indeed, among the 18 compounds identified here, montelukast [27], minocycline [28,29], and bexarotene [30] reportedly have beneficial effects in animal models of AD.

The four compounds we identified that selectively inhibited the binding of Aβ oligomers to EphB2 (DIH, BRO, CEP, and LEV) are approved for use in humans as treatments for migraine, Parkinson’s disease, and leucopenia, as well as for use as a contraceptive, respectively. Among these compounds, DIH, BRO, and CEP penetrate the blood–brain barrier [31–33]. Thus, our results suggest that these compounds, especially DIH, BRO, and CEP, may be safe and effective drugs for treatment of AD. On the other hand, these compounds required relatively high concentration to show their inhibitory effect on the binding of Aβ oligomers to EphB2. Therefore, we also consider the drug modification, using these approved medicines as leads.

As described above, the compounds identified in this study are expected to ameliorate Aβ oligomer-induced LTP impairment. EphB2 overexpression suppresses Aβ oligomer-induced neurotoxicity in cultured hippocampal neurons [34], and activation of EphB2 attenuates tau phosphorylation in a mouse model of AD [35]. Because both Aβ oligomer-induced neurotoxicity and

![Fig. 3. Effects of selected compounds on the binding of ephrinB2 to EphB2](image)

Each compound (100 μM) was added simultaneously with biotinylated ephrinB2-Fc to EphB2-coated wells, and the amount of bound biotinylated ephrinB2-Fc was determined. Values represent the mean ± SD (n = 3). **P < 0.01. LCA, lithocholic acid. Basically similar results were obtained in another independent experiment.
tau phosphorylation are important factors in AD pathology, the four compounds identified here may also prove useful for AD treatment through these mechanisms.

In conclusion, we screened for compounds that selectively inhibit the binding of Aβ oligomers to EphB2 from a library of approved drugs already in clinical use, and identified dihydroergotamine mesilate, bromocriptine mesilate, cephaparaine, and levonor- gestrel. We propose further analysis of these compounds as candidates for AD treatment.

Acknowledgements
This work was supported by JSPS KAKENHI Grant Number 15J02654, 24659037 and the Center of Innovation Program from Japan Science and Technology Agency.

Author contribution
KS, TI, and TM conceived and designed the project; KS and TA acquired the data; KS analyzed and interpreted the data; and KS, TI, and TM wrote the paper.

References
1 Haass C and Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer’s amyloid beta-peptide. Nat Rev Mol Cell Biol 8, 101–112.
2 Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA et al. (2008) Amyloid-beta protein dimers isolated directly from Alzheimer’s brains impair synaptic plasticity and memory. Nat Med 14, 837–842.
3 Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ and Selkoe DJ (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416, 535–539.
4 Malenka RC and Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44, 5–21.
5 Cisse M, Halabisky B, Harris J, Devidze N, Dubal DB, Sun B, Orr A, Lotz G, Kim DH, Hamto P et al. (2011) Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. Nature 469, 47–52.
6 Lauren J, Gimbel DA, Nygaard HB, Gilbert JW and Strittmatter SM (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. Nature 457, 1128–1132.
7 Freir DB, Nicoll AJ, Klyubin I, Panico S, Mc Donald JM, Risse E, Asante EA, Farrow MA, Sessions RB, Saibil HR et al. (2011) Interaction between prion protein and toxic amyloid beta assemblies can be therapeutically targeted at multiple sites. Nat Commun 2, 336.
8 Kullander K and Klein R (2002) Mechanisms and functions of Eph and ephrin signalling. Nat Rev Mol Cell Biol 3, 475–486.
9 Sheffler-Collins SI and Dalva MB (2012) EphBs: an integral link between synaptic function and synaptopathies. Trends Neurosci 35, 293–304.
10 Dalva MB, Takasu MA, Lin MZ, Shamah SM, Hu L, Gale NW and Greenberg ME (2000) EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. Cell 103, 945–956.
11 Grunwald IC, Korte M, Woller D, Wilkinson GA, Unsicker K, Lipp HP, Bonhoeffer T and Klein R (2001) Kinase-independent requirement of EphB2 receptors in hippocampal synaptic plasticity. Neuron 32, 1027–1040.
12 Henderson JT, Georgiou J, Jia Z, Robertson J, Elowe S, Roder JC and Pawson T (2001) The receptor tyrosine kinase EphB2 regulates NMDA-dependent synaptic function. Neuron 32, 1041–1056.
13 Simon AM, de Maturana RL, Ricobaraza A, Escribano L, Schiapparelli L, Cuadrado-Tejedor M, Perez-Mediavilla A, Avila J, Del Rio J and Frechilla D (2009) Early changes in hippocampal Eph receptors precede the onset of memory decline in mouse models of Alzheimer’s disease. J Alzheimers Dis 17, 773–786.
14 Qu M, Jiang J, Liu XP, Tian Q, Chen LM, Yin G, Liu D, Wang JZ and Zhu LQ (2013) Reduction and the intracellular translocation of EphB2 in Tg2576 mice and the effects of beta-amyloid. Neuropathol Appl Neurobiol 39, 612–622.
15 Mizushima T (2011) Drug discovery and development focusing on existing medicines: drug re-profiling strategy. J Biochem 149, 499–505.
16 Aimi T, Suzuki K, Hoshino T and Mizushima T (2015) Dextran sulfate sodium inhibits amyloid-beta oligomer binding to cellular prion protein. J Neurochem 134, 611–617.
17 Zhang JH, Chung TD and Oldenburg KR (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J Biomol Screen 4, 67–73.
18 Giorgio C, Hassan Mohamed I, Flammini L, Barocelli E, Incerti M, Lodola A and Tognolini M (2011) Lithocholic acid is an Eph-ephrin ligand interfering with Eph-kinase activation. PLoS ONE 6, e18128.
19 Deane R, Singh I, Sagare AP, Bell RD, Ross NT, LaRue B, Love R, Perry S, Paquette N, Deane RJ et al. (2012) A multimodal RAGE-specific inhibitor reduces amyloid beta-mediated brain disorder in a mouse model of Alzheimer disease. J Clin Invest 122, 1377–1392.
20 Risse E, Nicoll AJ, Taylor WA, Wright D, Badoni M, Yang X, Farrow MA and Collinge J (2015) Identification of a compound that disrupts binding of amyloid-beta to the prion protein using a novel fluorescence-based assay. *J Biol Chem* **290**, 17020–17028.

21 Fu AK, Hung KW, Huang H, Gu S, Shen Y, Cheng EY, Ip FC, Huang X, Fu WY and Ip NY (2014) Blockade of EphA4 signaling ameliorates hippocampal synaptic dysfunctions in mouse models of Alzheimer’s disease. *Proc Natl Acad Sci USA* **111**, 9959–9964.

22 Wang SD, Rath P, Lal B, Richard JP, Li Y, Goodwin CR, Laterra J and Xia S (2012) EphB2 receptor controls proliferation/migration dichotomy of glioblastoma by interacting with focal adhesion kinase. *Oncogene* **31**, 5132–5143.

23 Farshchian M, Nissinen L, Siljamaki E, Riihila P, Toriseva M, Kivisaari A, Ala-Aho R, Kallajoki M, Verajankorva E, Honkanen HK et al. (2015) EphB2 promotes progression of cutaneous squamous cell carcinoma. *J Invest Dermatol* **135**, 1882–1892.

24 Kudo W, Lee HP, Zou WQ, Wang X, Perry G, Zhu X, Smith MA, Petersen RB and Lee HG (2012) Cellular prion protein is essential for oligomeric amyloid-beta-induced neuronal cell death. *Hum Mol Genet* **21**, 1138–1144.

25 Kim T, Vidal GS, Djurisic M, William CM, Birnbaum ME, Garcia KC, Hyman BT and Shatz CJ (2013) Human LilrB2 is a beta-amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer’s model. *Science* **341**, 1399–1404.

26 Kam TI, Song S, Gwon Y, Park H, Yan JJ, Im I, Choi JW, Choi TY, Kim J, Song DK et al. (2013) FcγammaR1B mediates amyloid-beta neurotoxicity and memory impairment in Alzheimer’s disease. *J Clin Invest* **123**, 2791–2802.

27 Lai J, Hu M, Wang H, Long Y, Miao MX, Li JC, Wang XB, Kong LY and Hong H (2014) Montelukast targeting the cysteinyl leukotriene receptor 1 ameliorates Abeta1-42-induced memory impairment and neuroinflammatory and apoptotic responses in mice. *Neuropharmacology* **79**, 707–714.

28 Choi Y, Kim HS, Shin KY, Kim EM, Kim M, Park CH, Jeong YH, Yoo J, Lee JP, Chang KA et al. (2007) Minocycline attenuates neuronal cell death and improves cognitive impairment in Alzheimer’s disease models. *Neuropsychopharmacology* **32**, 2393–2404.

29 Ryu JK, Franciosi S, Sattayapraser P, Kim SU and McLarnon JG (2004) Minocycline inhibits neuronal death and glial activation induced by beta-amyloid peptide in rat hippocampus. *Glia* **48**, 85–90.

30 Cramer PE, Cirrito JR, Wesson DW, Lee CY, Karlo JC, Zinn AE, Casali BT, Restivo JL, Goebel WD, James MJ et al. (2012) ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models. *Science* **335**, 1503–1506.

31 Wang Y, Aun R and Tse FL (1998) Brain uptake of dihydroergotamine after intravenous and nasal administration in the rat. *Biopharm Drug Dispos* **19**, 571–575.

32 Friis ML, Paulson OB and Hertz MM (1979) Transfer of bromocriptine across the blood-brain barrier in man. *Acta Neurol Scand* **59**, 88–95.

33 Okamoto M, Ono M and Baba M (2001) Suppression of cytokine production and neural cell death by the anti-inflammatory alkaloid cepharanthine: a potential agent against HIV-1 encephalopathy. *Biochem Pharmacol* **62**, 747–753.

34 Geng D, Kang L, Su Y, Jia J, Ma J, Li S, Du J and Cui H (2013) Protective effects of EphB2 on Abeta1-42 oligomer-induced neurotoxicity and synaptic NMDA receptor signaling in hippocampal neurons. *Neurochem Int* **63**, 283–290.

35 Jiang J, Wang ZH, Qu M, Gao D, Liu XP, Zhu LQ and Wang JZ (2015) Stimulation of EphB2 attenuates tau phosphorylation through PI3K/Akt-mediated inactivation of glycogen synthase kinase-3beta. *Sci Rep* **5**, 11765.