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# Synthesis and evaluation of 2-pyridylbenzothiazole, 2-pyridylbenzoxazole and 2-pyridylbenzofuran derivatives as $^{11}$C-PET imaging agents for β-amyloid plaques

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ABSTRACT

The syntheses and SAR of new series of β-amyloid binding agents are reported. The effort to optimize signal-to-background ratios for these ligands are described. Compounds **8, 21** and **30** displayed desirable lipophilicity and pharmacokinetic properties. Compounds **8** and **21** were evaluated with in vitro autoradiographic studies and in vivo in APP/PS1 transgenic mice. It is shown that it was possible to increase the signal-to-background ratios compared to PIB **1**, as demonstrated by compounds **8** and **21**.



Alzheimer's disease (AD) is a neurodegenerative brain disorder characterized clinically by progressive decline of cognitive function. Pathologically, AD is characterised by amyloid plaques,[1] containing Aβ peptide(s), and by neurofibrillary tangles (NFTs) containing hyper-phosphorylated tau protein. Amyloid plaque accumulates prior to the onset of clinical symptoms. Detection of amyloid pathology early in disease progression, even before a clinical diagnosis of AD can be established, is therefore a key objective of current research in AD diagnosis. Biochemical measures of Aβ and tau in CSF have been shown to have utility as potential diagnostic tools, but there is also a need for Aβ plaque specific binding agents that can be used as PET (positron emission tomography) ligands for early diagnosis and monitoring of AD progression in the living brain.

Several types of β-amyloid imaging agents have been synthesized and evaluated including PIB **1**,[2] SB-13 **2**,[3] IMPY **3**[4] and FDDNP **4**[5] (Fig. 1). The reporting of several additional core structures that are not mutually displaceable, suggests that there are a number of different binding sites on β-amyloid in the Aβ plaques of AD patients.[6] The most examined compound PIB **1** has been tested clinically and, hence demonstrated to be a potential biomarker for the visualization of Aβ plaques in AD brains with PET.[2b,7] However, in order to increase the sensitivity to allow for even lower levels of Aβ plaques to be detected, and thus to monitor β-amyloid

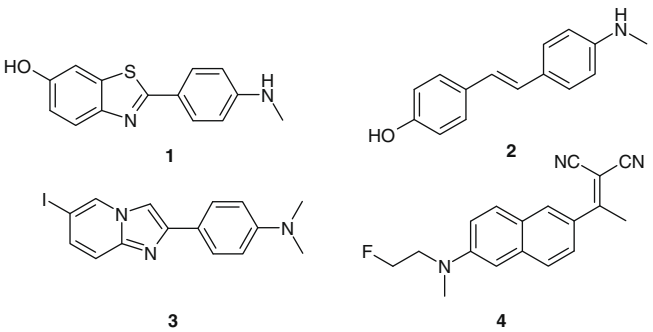


**Figure 1.** Examples of reported β-amyloid imaging agents.

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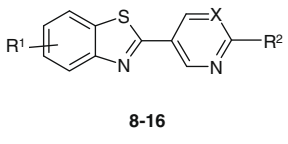


| | R¹ | R² | X |
|---|---|---|---|
| **8** | 6-OH | NHMe | C |
| **9** | 6-OMe | NHMe | C |
| **10** | 6-OH | $NMe_2$ | C |
| **11** | 6-OMe | $NMe_2$ | C |
| **12** | 5-OH | NHMe | C |
| **13** | 6-OH | $NMe_2$ | N |
| **14** | 6-OH | NHMe | N |
| **15** | 6-$CONH_2$ | NHMe | C |
| **16** | 6-NHMe | $CONH_2$ | C |

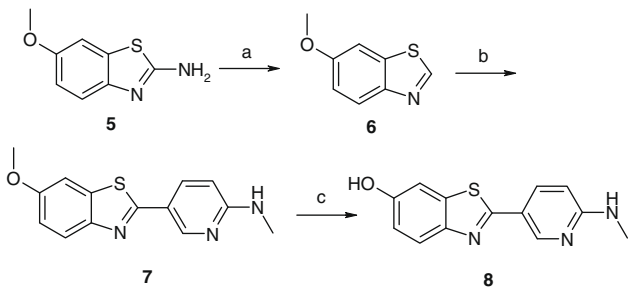
**8-16**

**Figure 2.** Synthesized 2-heteroarylbenzothiazoles.

lowing therapies with higher sensitivity, the non-specific binding of Aβ PET ligands have to be minimized. We envisaged that by decreasing the lipophilicity (as measured by *e* log *D*[8]) of potential ligands as compared to PIB **1**, the non-specific binding could be reduced, and the wash-out rate of non-specific binding in vivo could be increased (as measured by $t_{1/2}$[9] in β-amyloid devoid wild type rats). Three different compound series were explored with regard to (1) binding affinity to Aβ(1–40) fibrils, (2) lipophilicity (*e* log *D*), and (3) iv pharmacokinetic properties.

The 2-heteroarylbenzothiazoles were synthesized as exemplified in Scheme 1.[10] Commercially available 2-amino-6-methoxybenzothiazole **5** was deaminated by the use of *t*-butyl nitrite to afford 6-methoxybenzothiazole **6**. We found the method of direct arylation of 6-methoxybenzothiazoles using palladium catalysis useful also for bromopyridines.[11] Compound **6** was accordingly reacted with 2-methylamino-5-bromopyridine in the presence of Pd(*t*-$Bu_3P)_2$ and CuBr at 150 °C for 3 h to yield the 2-pyridylbenzothiazole **7** in moderate yield. Compound **7** was subsequently demethylated by employment of boron tribromide, to afford compound **8**.

In vitro binding affinities to human Aβ(1–40) fibrils were determined by competition radioligand binding assays in a fixed concentration of Aβ(1–40) fibrils (2 μM) and [$^3$H]PIB (3 nM), and over a concentration range of synthesized compounds.[12] Compound **10** (Fig. 2), having the highest binding affinity to Aβ(1–40) fibrils (Table 1), displayed a favourable *e* log *D* value and a similar half-life in brain ($t_{1/2}$) in comparison to compound **1**. Compound **8**, with a significant lower *e* log *D* and shorter half-life in brain ($t_{1/2}$) was judged to be promising and thus selected for autoradiographic evaluation. Compounds **9** and **11** both carrying a 6-methoxy substituent demonstrated unfavourably high *e* log *D*. Moving the hydroxyl group from 6- to the 5-position as in compound **12**, reduced the potency, *e* log *D* and $t_{1/2}$ in comparison to **8**, and the observed amount of compound present in brain 2 minutes after iv dosing was drastically reduced. The pyrimidines **13** and **14** were less potent than the corresponding pyridines. Albeit compound **13** was still quite potent and the *e* log *D* and the uptake were acceptable, the compound displayed an unfavourable long half-life. The carboxamides **15** and **16** were surprisingly potent but the former

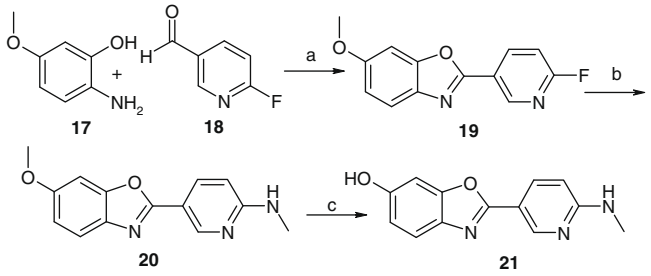


**Scheme 1.** Reagents and conditions: (a) *t*-butylnitrite, THF, 60 °C, 42%; (b) 2-methylamino-5-bromopyridine, $Cs_2CO_3$, Pd(*t*-$Bu_3P)_2$, CuBr, DMF, 150 °C, 24%; (c) $BBr_3$, $CH_2Cl_2$, rt, 62%.

**Table 1**
$IC_{50}$ values, *e* log *D*[8] and iv PK properties[9] for compounds **8–16**

| Compound | $IC_{50}$[a] (nM) | *e* log *D* | $t_{1/2}$ (min) | Dose in brain at 2 min% |
|---|---|---|---|---|
| **1** (0)[b] | 27 | 2.8 | 11.6 | 1.4 |
| **8** (+) | 58 | 1.8 | 6.8 | 1.0 |
| **9** (−) | 58 | 3.2 | 9.7 | 1.9 |
| **10** (−) | 19 | 2.4 | 11.9 | 1.0 |
| **11** | 81 | 4.2 | 17.7 | 1.9 |
| **12** | 74 | 1.5 | 4.8 | 0.2 |
| **13** | 41 | 2.2 | 17.0 | 1.9 |
| **14** | 267 | 0.9 | 8.4 | 0.9 |
| **15** | 85 | 0.5 | 8.4 | 0.2 |
| **16** | 35 | 1.7 | 8.8 | 0.9 |

[a] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10\%$.
[b] The sign (−), (0), or (+), indicates the relative non-specific binding level of the corresponding [$^3$H]-labelled compound.



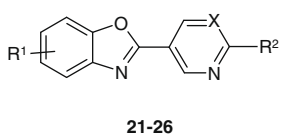
**Scheme 2.** Reagents and conditions: (a) (1) $Et_3N$, MeOH; (2) DDQ, $CH_2Cl_2$, 41%; (b) $MeNH_2$, EtOH, 50 °C, 100%; (c) $BBr_3$, $CH_2Cl_2$, 55%.

showed a very low brain uptake, probably due to the relatively low lipophilicity.

The 2-heteroarylbenzoxazoles were synthesized as exemplified in Scheme 2.[13] Oxidative cyclization of a phenolic Schiff base, derived from the condensation of 2-hydroxy-6-methoxyaniline **17** with 2-fluoro-5-formyl-pyridine **18**, using DDQ as oxidant afforded the 2-pyridylbenzoxazole **19** in moderate yield.[14] Subsequent substitution with methyl amine in ethanol gave compound **20** in quantitative yield. Selective demethylation of **20** with boron tribromide at 0 °C yielded compound **21**.

The benzoxazole derivatives (Fig. 3) were generally less potent than the corresponding benzothiazoles (Table 2). The compound with the highest binding affinity amongst the pyridines in this series was the 6-hydroxy-dimethylamino derivative **24**, in analogy with the corresponding benzothiazole **10**. A signal-to-background evaluation of selected compounds from this series was warranted due to the general low lipophilicity. Pyrimidine **26** was relatively potent and displayed more favourable properties than the corresponding benzothiazole **13**.

The 2-heteroarylbenzofurans were synthesized as exemplified in Scheme 3.[15] Commercially available 5-methoxybenzofuran **27** was lithiated with BuLi at −78 °C, before treatment with triisopropylborate to yield boronic acid **28**. Compound **28** was subsequently reacted with 2-methylamino-5-bromopyridine under Suzuki reaction conditions to afford the 2-pyridylbenzofuran **29**. Subsequent demethylation of **29** with boron tribromide yielded compound **30**.



| | R¹ | R² | X |
|---|---|---|---|
| **21** | 6-OH | NHMe | C |
| **22** | 6-OMe | $NMe_2$ | C |
| **23** | 6-OMe | NHMe | C |
| **24** | 6-OH | $NMe_2$ | C |
| **25** | 5-OH | $NMe_2$ | C |
| **26** | 6-OH | $NMe_2$ | N |

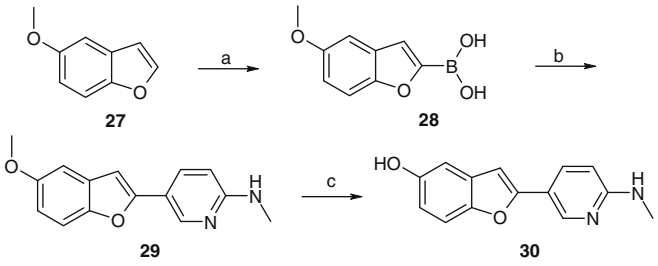
**21-26**

**Figure 3.** Synthesized 2-heteroarylbenzoxazoles.

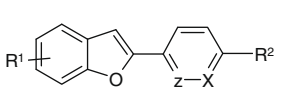
**Table 2**
$IC_{50}$ values, *e* log *D*[8] and iv PK properties[9] for compounds **21–26**

| Compound | $IC_{50}$[a] (nM) | *e* log D | $t_{1/2}$ (min) | Dose in brain at 2 min% |
|---|---|---|---|---|
| **21** (+)[b] | 182 | 1.3 | 7.2 | 0.5 |
| **22** (−) | 204 | 3.7 | 9.7 | 2.1 |
| **23** | 185 | 2.7 | 9.3 | 1.0 |
| **24** (0) | 85 | 1.9 | 10.0 | 1.0 |
| **25** | 137 | 2.2 | 10.9 | 1.9 |
| **26** (0) | 75 | 1.8 | 10.0 | 1.6 |

[a] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10\%$.
[b] The sign, (−), (0) or (+), indicates the relative non-specific binding level of the corresponding [$^3$H]-labelled compound.




**Scheme 3.** Reagents and conditions: (a) BuLi, THF, −78 °C, triisopropylborate, 92%; (b) $Pd(PPh_3)_2Cl_2$ / $Et_3N$, 2-methylamino-5-bromopyridine, EtOH, microwave, 140 °C, 37%; (c) $BBr_3$, $CH_2Cl_2$, 0 °C to rt, 40%.



| | R¹ | R² | X | Z |
|---|---|---|---|---|
| **30** | 5-OH | NHMe | N | C |
| **31** | 5-OMe | NHMe | N | C |
| **32** | 5-OMe | $CONH_2$ | N | C |
| **33** | 5-OMe | $CONH_2$ | C | N |

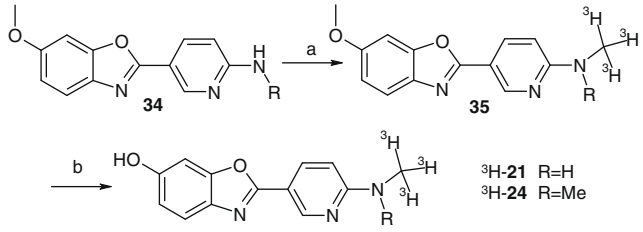
**30-33**

**Figure 4.** Synthesized 2-heteroarylbenzofuranes.

**Table 3**
$IC_{50}$ values, *e* log *D*[8] and iv PK properties[9] for compounds **30–33**

| Compound | $IC_{50}$[a] (nM) | *e* log *D* | $t_{1/2}$ (min) | Dose in brain at 2 min% |
|---|---|---|---|---|
| **30** | 45 | 2.0 | 7.7 | 0.6 |
| **31** | 66 | 3.8 | n.d. | n.d. |
| **32** | 60 | 1.7 | 10 | 1.45 |
| **33** | 33 | 2.8 | 14 | 1.9 |

[a] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10\%$.




**Scheme 4.** Reagents and conditions: (a) [$^3$H]MeI, NaH, DMF, 60 °C, 1 h, 44% for R = H; (b) sodium thiophenoxide, *N*-methylpyrrolidinone, microwave, 30 min, 250 °C, 82% for R = H.

The benzofuran derivatives (Fig. 4) generally showed quite high binding affinity to Aβ(1–40) fibrils (Table 3). Compound **30** displayed a favourable *e* log *D* and half-life values in comparison to compound **1**. Both of the carboxamides **32** and **33** were potent binders, interestingly the latter displayed a significant higher *e* log *D* value in comparison to the former. This was reflected in a longer $t_{1/2}$ and in a higher amount of compound **33** entering the brain.

The corresponding [$^3$H]-NMe labelled derivatives were synthesized as exemplified in Scheme 4.[13] Deprotonation of **34** with sodium hydride and alkylation with [$^3$H]MeI in DMF afforded the [$^3$H]-labelled compound **35**. Subsequent demethylation with sodium thiophenoxide in *N*-methylpyrrolidinone at elevated temperature gave [$^3$H]-**21** in good yield.

In vitro binding studies employed for the comparison of [$^3$H]-compound **1** with the [$^3$H]-labelled compounds **8–11**, **21, 22, 24** and **26**, were performed on dissected cortical tissue sections from AD patients, as shown in Figure 5 for [$^3$H]-**1, 8** and **21**.[16] The results from these experiments demonstrated that the ligands display similar binding patterns.

There was an evident difference in non-specific binding amongst the [$^3$H]-labelled compounds. Comparison of non-specific binding as defined by saturation with unlabelled compound **1** (1 μM) in AD brain sections showed that [$^3$H]-**8** (1 nM) displayed significantly lower levels of non-specific binding as compared to [$^3$H] **1** (1nM) (Fig. 6).

The analysis results of signal-to-background ratio in AD tissue for [$^3$H]-labelled compounds are shown in Tables 1 and 2. [$^3$H]-**1** is by definition '0' and compounds having better (higher) signal-to-background ratios are identified as '+' and consequently unfavourable ratios (lower) are defined as '−'. Compounds **8**, **21 24** and **26** with low non-specific binding (0, +) had as anticipated also lower *e* log *D* values and shorter half-life than compound **1**. It can also be concluded that, apart from a low lipophilicity and a short

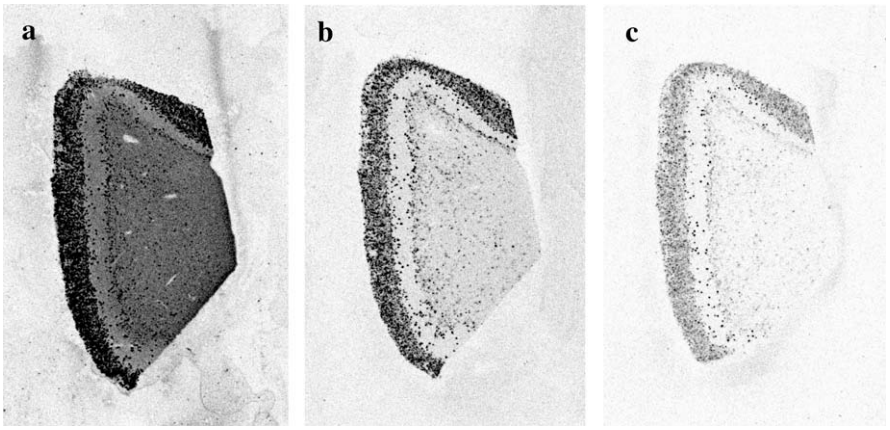



**Figure 5.** In vitro autoradiographic comparison of [$^3$H]-compounds (3 nM) binding in AD tissue. (a) [*N-Methyl*-$^3H_3$]-2-[4-(methylamino)phenyl]-6-hydroxybenzothiazole **1**; (b) [*N-Methyl*-$^3H_3$]-2-(6-methylamino-pyridin-3-yl)-6-hydroxy- benzothiazole **8**; (c) [*N-Methyl*-$^3H_3$]-2-(6-methylamino-pyridin-3-yl)-6-hydroxybenzoxazole **21**.

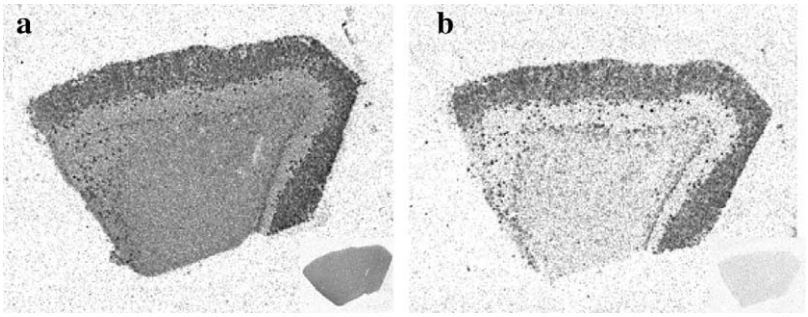



**Figure 6.** In vitro autoradiographic comparison of non-specific binding. (a) [$^3$H]-**1** (1 nM), (b) [$^3$H]-**8** (1 nM) binding in AD tissue. Both ligands display high levels of cortical binding but [$^3$H]-**8** shows substantially lower background levels in subcortical regions and in tissue slices exposed to 1 μM of unlabelled PIB **1** (as shown in the inserts).

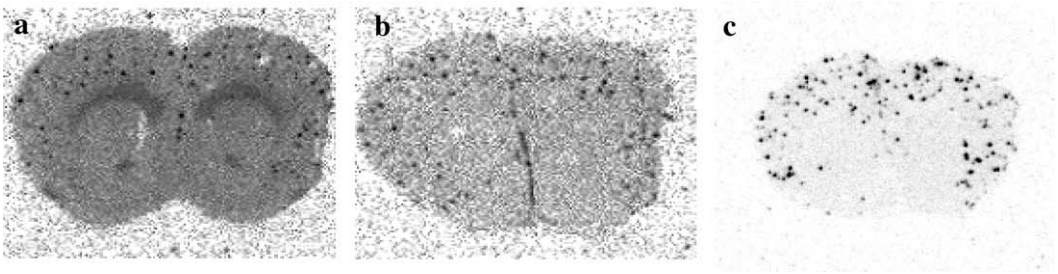



**Figure 7.** Autoradiographic binding (ex vivo) to plaques in brain sections from APP/PS1 mice after iv administration of (a) [$^3$H]-**1**, (b) [$^3$H]-**8**, (c) [$^3$H]-**21**.

half-life, it is essential that the molecules have a hydrogen bond donor on both the left-hand and right-hand side, probably in order to specifically bind to the amyloid plaques, as can be seen in the comparison of **8** and **21** with **10, 24** and **26**. These specific hydrogen bond donor interactions with Aβ plaques contribute to the best signal-to-background ratios (+) for compounds **8** and **21**. The benzofuran series was not evaluated in these autoradiographic studies but compound **30** possessed the prerequisite properties for a selective PET ligand. The benzofuran, together with the benzothiazole and the benzoxazole series, are presently under investigation as [$^{18}$F]-ligands and these results are going to be described in a subsequent paper.

Compounds **1**, **8** and **21** were evaluated for plaque binding in APP/PS1 transgenic mice. Intravenous injection of one of the corresponding [$^3$H]-labelled compounds (1 mCi) followed by ex vivo binding autoradiographic analysis in brain sections, after 10 min exposure of the compound in vivo, showed excellent plaque staining for all three compounds (Fig. 7).[17] These ex vivo binding studies showed that the superior signal-to-background ratio of compounds **8** and **21** also translates to the in vivo case.

In order to be able to detect low levels of Aβ plaques and monitor AD disease modifying therapies the non-specific binding of PET ligands have to be minimized. Hence three different compound series have been evaluated as potential biomarkers for Aβ plaques. We have shown that it is possible to increase the signal-to-background ratios in comparison to PIB **1**, as demonstrated by compounds **8** and **21**. Furthermore we have confirmed that the lipophilicity and the wash-out rate (measured as the half-life in brain) are crucial properties for achieving a low non-specific background binding.

The specific binding to Aβ plaques is apparently dependent upon the compound ability to form hydrogen bonds with Aβ. It is essential that the molecules have a hydrogen bond donor both on the left-hand and right-hand side in order to specifically bind to amyloid plaques in AD brain tissue. The preclinical profile of compounds **8** and **21** in relation to the reference ligand PIB **1**, suggest that the corresponding $^{11}$C-labelled compounds **8** or **21** may enable PET-visualization of β-amyloid deposits in the living human brain with a higher sensitivity than the currently used PET ligands.

## Acknowledgements

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9. Intravenous (iv) cassette dosing (three test compounds and PIB) was used to compare the in vivo pharmacokinetic characteristics of the candidate compounds directly with PIB. All compounds were dissolved in a polyethylene glycol 400/dimethylamide/water mixture (40:40:20 v/v/v) at a concentration of 0.25 μmol/mL each, and administered to rats (slow bolus dose, 4 mL/kg, 3 rats per time point). Prior to decapitation, blood samples (400 μL) were collected from the tail vein 2 and 30 min after dosing, immediately placed on ice, and centrifuged within 30 min at 4 °C for 5 min at 2000*g* to obtain plasma. Brains were removed, homogenized in cold (4 °C) Ringer solution (1 part brain + 2 parts Ringer, w/v, ULTRA-Turrax T8 homogenizer, IKA, Staufen, Germany) and sonicated (Ultrasonic Processor UP200H, Hielscher Ultrasonics, Berlin, Germany). Plasma and brain samples were precipitated with acetonitrile and after mixing and centrifugation, the supernatant was diluted with mobile phase and analyzed by LC–MS–MS. At 2 min after drug administration, the percentage of the total dose in brain was calculated and values are reported in Tables 1–3. The half-life ($t_{1/2}$) of compounds in the rat brain was estimated from the 2 and 30 min concentration values.
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17. *Method for ex vivo autoradiography*: Autoradiography on brain sections from APP/PS1 mice 10 min after iv administration of 0.3 nmol/kg of [$^3$H]-labelled compound were performed (Ref. 12).