Structure−Activity Relationship of Imidazo[1,2-*a*]pyridines as Ligands for Detecting *β*-Amyloid Plaques in the Brain

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A series of novel *β*-amyloid (A*β*) aggregate-specific ligands, 2-(4′-dimethylaminophenyl)-6-iodoimidazo[1,2-*a*]pyridine, **16**(IMPY), and its related derivatives were prepared. An in vitro binding study with preformed A*β* aggregates showed that **16**(IMPY) and its bromo derivative competed with binding of 2-(4′-dimethylaminophenyl)-6-iodobenzothiazole, [^{125}I]**7**(TZDM), a known ligand for A*β* aggregates, with high binding affinities (K_i = 15 and 10 nM, respectively). In vitro autoradiography of brain sections of a transgenic mouse (Tg2576) with [^{125}I]**16**(IMPY) displayed high selective binding to amyloid-like structures, comparable to that observed by staining with thioflavin-S visualized under fluorescence. In vivo biodistribution after an intravenous injection of [^{125}I]**16**(IMPY) in normal mice showed a high initial brain uptake and fast washout, indicating a low background activity associated with this iodinated ligand. Taken together, the data suggests that [^{123}I]**16**(IMPY) may be useful for imaging A*β* aggregates in patients with Alzheimer's disease.

Introduction

Formation of *β*-amyloid (A*β*) plaques in the brain is a pivotal event in the pathology of Alzheimer's disease (AD). Significant circumstantial evidence suggests that fibrillary A*β* plaques consisting predominantly of aggregates of A*β*40 and A*β*42 peptides play a major role in AD pathogenesis.[1,2] The accumulation of A*β* plaques is now considered one of the most significant factors in AD. A*β* aggregate-specific probes for in vitro and in vivo studies of A*β* plaques are potentially important for diagnosis and monitoring of therapeutic effects of drugs aiming at lowering the A*β* burden in the brain by noninvasive imaging.[1,3] There are several potential benefits of imaging A*β* aggregates in the brain. The imaging technique will improve diagnosis by identifying potential patients with A*β* plaques in the brain, who are likely to have AD. It may also be useful to monitor the progression of the disease. When antiplaque drug treatments become available, imaging A*β* plaques in the brain will be useful for monitoring treatment. The in vivo imaging of A*β* plaques may provide information to understand better AD symptoms vs A*β* burden in the brain. Such information is very difficult to obtain in the living human brain by other imaging methods.

Currently, small-molecule-based diagnostic imaging agents for A*β* aggregates in the brain can be labeled with a suitable isotope for PET or SPECT imaging. ^{99m}Tc ($T_{1/2}$ = 6 h; 140 keV) and ^{123}I ($T_{1/2}$ = 13 h; 159 keV) are routinely used for single photon emission computed tomography (SPECT), whereas ^{11}C ($T_{1/2}$ = 20 min; 511 keV) and ^{18}F ($T_{1/2}$ = 110 min; 511 keV) are commonly used for positron emission tomography (PET). Development of A*β* aggregate-specific imaging agents is often based on highly conjugated dyes, such as Congo Red (CR) and Chrysamine G (CG) (Chart 1). Thioflavins-S and -T, as well as CR, have been used in fluorescent staining of plaques and tangles in postmortem AD brain sections. More abbreviated derivatives of CG, styrylbenzenes (SB), such as **1**,[4] **2**,[5] **3**,[6] and **4**[7] (Chart 1), have been reported. Because ionic and bulky molecules are not able to penetrate intact blood−brain barrier (BBB), attempts to develop labeled CG and CR derivatives as imaging agents were, in general, unsuccessful[8−10] even though these molecules are highly conjugated, show high binding affinity, and display some lipophilicity.

To achieve a high brain penetration, it is normally considered prudent to use neutral, small, and lipophilic compounds. Interestingly, a neutral and highly lipophilic probe, [^{18}F]**5**(FDDNP) (Chart 1), for binding tangles and plaques has been reported. Preliminary imaging study in humans appears to suggest that [^{18}F]-**5**(FDDNP) showed a higher retention in regions of brain suspected of having tangles and plaques.[11,12] A neutral thioflavin derivative, **6**(BTA-1) (Chart 1), was recently reported. In an in vitro binding assay with A*β*40 aggregates, **6** showed an excellent affinity (K_i = 11 nM).[13] When [^{11}C]**6** was injected (iv) into normal mice, it showed excellent brain penetration.[13−15] Alternative iodinated and neutral thioflavins, [^{125}I]**7**(TZDM) and **8**, were prepared in our laboratory (Chart 1). These neutral benzothiazole derivatives showed excellent binding affinities, with K_d values of <1 nM for aggregates of A*β*40. Interestingly, under a competitive-binding assaying condition, different binding sites on A*β* aggregates,

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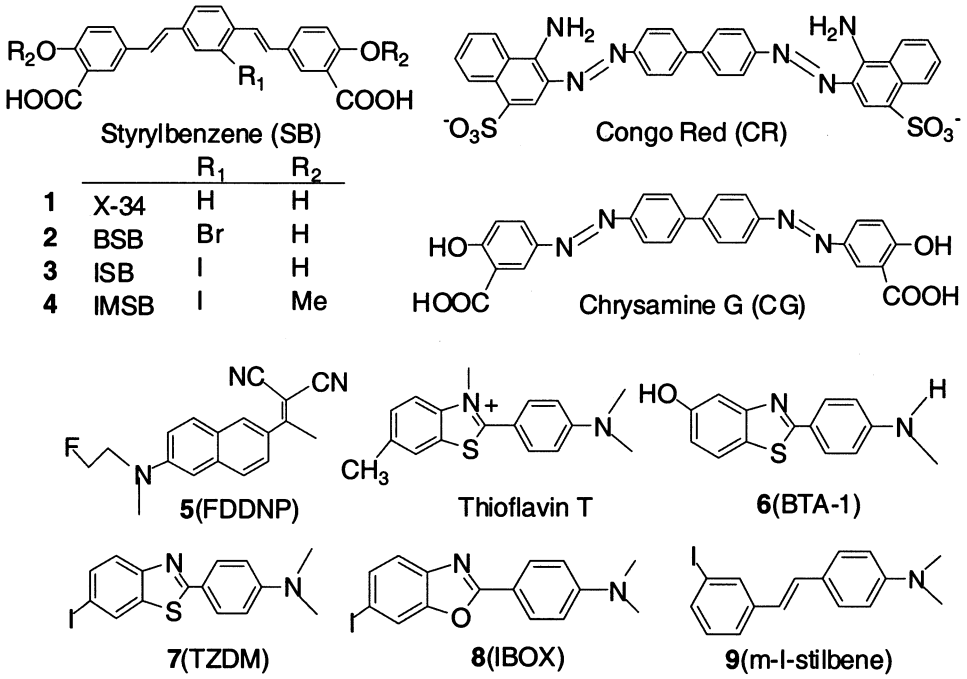
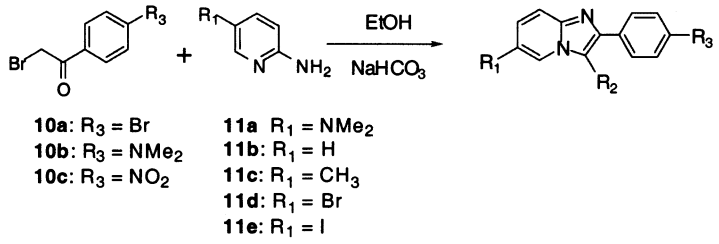
Chart 1



		R_1	R_2
1	X-34	H	H
2	BSB	Br	H
3	ISB	I	H
4	IMSB	I	Me

Scheme 1



10a: R_3 = Br
10b: R_3 = NMe_2
10c: R_3 = NO_2

11a R_1 = NMe_2
11b: R_1 = H
11c: R_1 = CH_3
11d: R_1 = Br
11e: R_1 = I

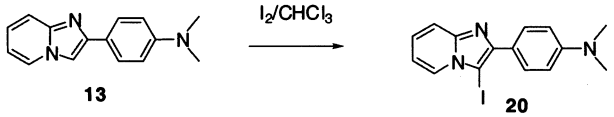
	R_1	R_2	R_3	
12a	H	H	NO_2	Na_2S/H_2O
12b	H	H	NH_2	1. CH_3CO_2CHO; 2. LAH
12c	H	H	NHMe	$(CH_2O)_n$, $NaBH_4$, TFA-THF
13	H	H	NMe_2	
14a	CH_3	H	NO_2	Na_2S/H_2O
14b	CH_3	H	NH_2	1. CH_3CO_2CHO; 2. LAH
14c	CH_3	H	NHMe	$(CH_2O)_n$, $NaBH_4$, TFA-THF
15	CH_3	H	NMe_2	
16	I (IMPY)	H	NMe_2	
17	Br	H	NMe_2	
18	CH_3	H	Br	
19	NMe_2	H	Br	

which are mutually exclusive, were observed for styrylbenzenes (SB) and thioflavins or benzothiazoles (TZ).[7] It is observed that **1**−**4** bind to the SB sites with high affinity, while **7**(TZDM) and **8** bind to the TZ sites.

Significantly, biodistribution studies in normal mice after an iv injection showed that [^{125}I]**7** and **8** exhibited excellent brain uptake and retention, which were much higher than those of [^{125}I]**3** and **4**.[7,16] To further modify the benzothiazole series of compounds, a group of stilbenes was prepared.[17] Among the stilbenes, **9** (Chart 1) displayed an excellent binding affinity. Binding of [^{125}I]**9** to aggregates of Aβ40 is saturable, and the dissociation constant (K_d) is 0.19 nM, which is comparable to that observed for **7**.[7] In normal mice, [^{125}I]**9** showed excellent brain penetration (>1% dose/g after an iv injection in mice).[17] On the basis of known compounds showing good binding affinity to Aβ aggregates, it can be hypothesized that they all share a common structural feature: all of the active compounds contain either an *N*-methylamino- or *N*,*N*-dimethylaminophenyl group on one end of the molecule. The structural feature required for binding to Aβ aggregates appears to be simple and unique.

In the search for novel ligands for Aβ aggregates based on the benzothiazole ring system, it was observed

Scheme 2



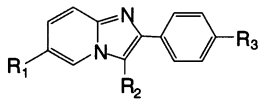
13 **20**

that imidazo[1,2-*a*]pyridine derivatives containing the desired *N*,*N*-dimethylaminophenyl group, such as **16**-(IMPY), have binding properties similar to those of the other iodinated benzothiazole ligands. It shares the same critical structural component, the *N*,*N*-dimethylaminophenyl group, known to have good binding affinity. Reported herein are the synthesis and the structure−activity relationship of this series of derivatives. Initial in vivo and in vitro characterizations of [^{125}I]**16**(IMPY) as an Aβ aggregate-specific imaging agent are reported.

Results and Discussion

Chemistry. Syntheses of imidazo[1,2-*a*]pyridine derivatives were achieved by reactions shown in Schemes 1−3. The initial formation of the imidazo[1,2-*a*]pyridine ring was readily accomplished by a fusion reaction between a bromoketone[18] and 2-aminopyridines under

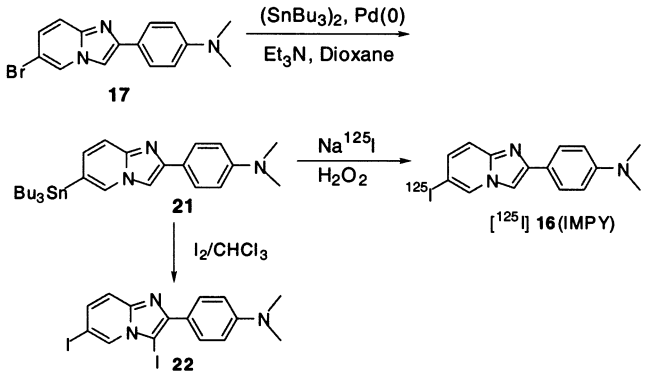
Table 1. Inhibition Constants (K_i, nM)[a] and Spectroscopic Properties[b] of IMPY and Its Derivatives



compd	R_1	R_2	R_3	K_i (nM)	λ (nm), log ϵ	ϕ (in EtOH) (λ_{ex} [nm], λ_{em} [nm])[b]
12c	H	H	NHMe	>1000	355, 4.14 281, 4.06 217, 4.31	0.24 (345, 446)
13	H	H	NMe_2	>2000	336, 4.25 277, 4.38	0.25 (337, 450)
14c	CH_3	H	NHMe	>2000	not measured	not measured
15	CH_3	H	NMe_2	242 ± 20	not measured	not measured
16(IMPY)	I	H	NMe_2	15 ± 5	356, 4.26 285, 4.44 234, 4.52	0.039 (339, 446)
17	Br	H	NMe_2	10.3 ± 1.2	355, 4.21 283, 4.41 229, 4.50	0.071 (350, 460)
18	CH_3	H	Br	638 ± 30	323, 3.97 253, 4.55 210, 4.38	0.20 (320, 370)
19	NMe_2	H	Br	339 ± 40	327, 3.90 276, 4.35	0.064 (330, 414)
20	H	I	NMe_2	>2000	340, 4.14 277, 4.38 227, 4.48	0.0043 (340, 440)
22	I	I	NMe_2	>2000	358, 4.10 286, 4.35 246, 4.40	too low to be measured
7(TZDM)				0.9 ± 0.2	366, 4.29	0.21 (360, 425)

[a] Inhibition constants were measured by in vitro binding assay using preformed Aβ40 aggregates and [^{125}I]**7** (TZDM) as the ligand. [b] Referenced to a quinine hydrochloride solution in 0.1 N H_2SO_4 (ϕ = 0.55).

Scheme 3



a mild basic condition to give the desired imidazo[1,2-*a*]pyridines in good yields (Scheme 1). The fusion reaction shown in Scheme 1 is versatile, and it has been applied in the synthesis of derivatives bearing various substitution groups on the ring system. The starting materials, bromoketone and 2-aminopyridine, are generally available commercially, whereas 2-amino-5-(*N*,*N*-dimethylamino)pyridine was prepared by a known method reported previously.[19] The nitro derivatives, **12a** and **14a**, where R_3 = NO_2, were readily reduced by sodium sulfide to amino derivatives, **12b** and **14b**,[20,21] in excellent yields. Subsequently, the amino group of **12b** and **14b** was converted to an *N*-methylamino group by a selective monomethylation reaction using formic acetic anhydride at ambient temperatures for 30 min, followed by a reduction reaction in the presence of $LiAlH_4$. The monomethylated **12c** and **14c** were again methylated by paraformaldehyde and sodium borohydride to give the *N*,*N*-dimethylamino derivatives **13** and **15** in good yields. Treatment of compound **13** with iodine in chloroform led to the formation of the 3-iodo derivative, **20** (Scheme 2). Apparently, the 3-position is the most electron-rich position for an iodination reaction. This sensitivity for electrophilic substitution will play an important role in the iodination of the series of compounds.

The starting material for radioiodination, the tri-*n*-butyltin derivative **21**, was prepared with a palladium-catalyzed reaction (Scheme 3). Preparation of radioiodinated [^{125}I]**16**(IMPY) was carried out by an iododestannylation reaction catalyzed by hydrogen peroxide (Scheme 3). The desired tracer, [^{125}I]**16**(IMPY), was readily purified by HPLC (radiochemical yield 20−60%, radiochemical purity >95%). To characterize the tracer fully, a comparable reaction in a higher chemical amount was attempted by reacting **21** with "cold" iodine in chloroform. Surprisingly, the product from this reaction showed no binding affinity at all. Upon closer inspection, it was found that the "cold" iododestannylation reaction produced a 3,6-diiodo compound, **22**, instead of **16**(IMPY). An alternative synthesis by reaction of **10b** with 2-amino-5-iodopyridine, **11e**, yielded **16**(IMPY) unambiguously.

Under a no-carrier-added condition, the iododestannylation reaction produced only the expected monoiodo compound, **16**(IMPY). It was reported previously that the imidazo[1,2-*a*]pyridine ring will readily undergo an electrophilic substitution reaction at the 3-position.[22] The result showed that, even under a very mild reaction condition, I_2 in chloroform, the electrophilic substitution reaction at the 3-position will take place. The iodination reaction of the imidazo[1,2-*a*]pyridine ring produced

Table 2. Biodistribution of Radioactivity in Normal Mice (ICR) after an Intravenous Injection of [^{125}I]**16**(IMPY) (% Dose/Organ)[a]

organ	2 min	30 min	1 h	2 h	6 h	24 h
blood	6.41 ± 0.77	2.44 ± 0.36	2.50 ± 0.11	1.82 ± 0.21	1.40 ± 0.27	0.18 ± 0.02
heart	0.79 ± 0.14	0.16 ± 0.02	0.12 ± 0.02	0.08 ± 0.01	0.04 ± 0.01	0.01 ± 0.00
muscle	13.8 ± 3.44	6.08 ± 0.59	5.03 ± 1.03	2.96 ± 0.84	1.46 ± 0.42	0.27 ± 0.11
lung	1.56 ± 0.33	0.31 ± 0.07	0.34 ± 0.08	0.20 ± 0.05	0.12 ± 0.05	0.05 ± 0.03
kidney	4.75 ± 0.49	1.51 ± 0.27	1.17 ± 0.29	0.53 ± 0.05	0.25 ± 0.05	0.05 ± 0.01
spleen	0.40 ± 0.06	0.09 ± 0.02	0.08 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.01 ± 0.00
liver	20.9 ± 2.63	6.32 ± 0.55	5.88 ± 0.85	2.90 ± 0.21	1.54 ± 0.08	0.61 ± 0.11
skin	5.72 ± 0.90	4.69 ± 1.06	4.28 ± 0.25	3.14 ± 0.51	2.19 ± 0.63	0.22 ± 0.06
brain	2.88 ± 0.25	0.26 ± 0.00	0.21 ± 0.03	0.14 ± 0.03	0.06 ± 0.02	0.02 ± 0.00

[a] Average of 3 or 4 mice per time point ± standard deviation.

very different results from other comparable fused ring systems, such as benzothiazole, **7**(TZDM).

Biological Studies. In an in vitro binding assay, **16**(IMPY) competed with [^{125}I]**7** (TZDM) binding to Aβ40 aggregates, showing an excellent binding affinity (K_i = 15.0 ± 5.0 nM, Table 1). A comparable binding affinity value was obtained for the corresponding bromo derivative, **17** (K_i = 10.3 ± 1.2 nM). Other modifications of **16**(IMPY) appeared to decrease the binding affinity to Aβ aggregates, as measured by in vitro competition with [^{125}I]**7** (Table 1). Elimination of the halogen groups at the 6-position (compounds **12** and **13**) reduced the binding affinity dramatically (K_i > 2000 nM); replacing the 6-halogen with a methyl group also reduced the binding significantly (K_i for **15** was 242 nM). It is also worth noting that the 6-methyl derivative, **18**, or the 6-(*N*,*N*-dimethlyamino)-2-(4′-bromophenyl) derivative, **19**, showed low binding affinities (K_i = 638 and 339 nM, respectively). The overall molecular size of **16**(IMPY) is similar to that of **7** or **9**. Similar to the findings reported previously,[21,23] the imidazo[1,2-*a*]pyridine compounds showed strong absorbance, with λ_{max} = 320−360 nm, with a quantum yield (ϕ value) of 3.97−4.26 (Table 1). The quantum yields (ϕ) of fluorescence were measured using quinine as a standard.[24] The ϕ values ranged from 0 to 0.25. The compounds containing halogens, **16**(IMPY), **17**, **20**, and **22**, showed the lowest values, whereas compounds **12c**, **13**, and **18** showed ϕ values of 0.20−0.25, comparable to that of **7** (ϕ = 0.21). The dramatic decrease in intensity of fluorescence upon introduction of halogens such as iodine or bromine is well known.[25] This series of compounds with halogen substitution at the 6-position in general exhibited a higher binding affinity toward the aggregates. It is important to note that the UV absorption and the fluorescent properties of this series of compounds are not coupled to the binding affinity toward the Aβ aggregates (Table 1). Apparently, the high fluorescent properties needed for staining the plaques in vitro is not a prerequisite for binding to the Aβ aggregates.

Brain sections of a 16-month-old Tg2576 mouse were labeled with [^{125}I]**16**(IMPY). A distinctive plaque-labeling pattern was clearly visualized with a low background activity (Figure 1). When the same brain section was stained with the fluorescent dye, thioflavin-S, the same Aβ plaques showed prominent fluorescent labeling, consistent with the results of an autoradiogram.

A biodistribution study in normal mice after an iv injection showed that [^{125}I]**16**(IMPY) exhibited excellent brain uptake (2.9% ID/organ at 2 min) and fast washout (0.2% ID/organ at 1 h), which is highly desirable for Aβ plaque imaging agents (Table 2). At 6 and 24 h, there was essentially no remaining brain uptake. The blood

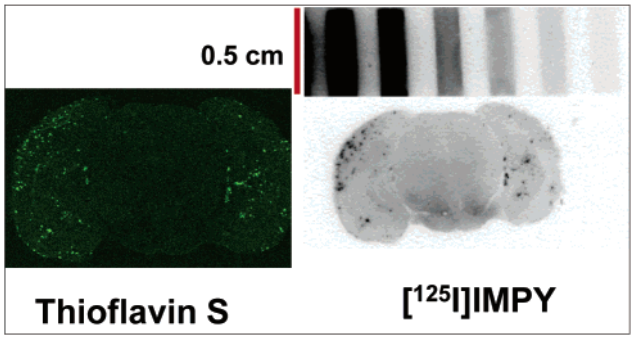


Figure 1. Film autoradiogram of brain sections from a Tg2576 mouse (right) labeled with [^{125}I]**16**(IMPY). The plaques were confirmed with an adjacent section fluorescently stained with thioflavin-S (left).

levels are relatively low at all time points measured. The tracer seems to distribute in high blood flow areas, such as liver, kidney, muscle, and skin (Table 2). The partition coefficient (PC) of **[**^{125}I]**16** is 100 (1-octanol/buffer),[26] which is comparable to that of [^{125}I]**7** (PC = 70).[7] A relatively high lipophilicity may be critical for the initial brain penetration by a simple diffusion mechanism. The most important feature of this novel ligand, [^{125}I]**16**(IMPY), is the faster rate of washout from the normal brain. This fast rate is due to a lack of trapping mechanisms in the normal brain (no Aβ plaques). As expected, the Aβ plaques in the brain of a Tg2576 mouse can be distinctly visualized and detected in vivo by using [^{125}I]**16**(IMPY).[26]

In summary, this novel imidazo[1,2-*a*]pyridine derivative, **[**^{125}I]**16**(IMPY), is a radioiodinated compound showing excellent in vitro binding and in vivo biodistribution data. Derivatives of **16**(IMPY) generally show lower binding affinity, suggesting that highly selective substitution groups are needed on specific locations of the imidazo[1,2-*a*]pyridine ring. The in vivo biodistribution of [^{125}I]**16**(IMPY) in normal mice showed excellent brain uptake and washout. The initial observation in normal mice is confirmed in the transgenic mice engineered to develop excess Aβ plaques. It appears that this ligand will have highly desirable properties: fast kinetics of high initial brain uptake and rapid washout in the non-Aβ plaque-containing areas. Tracers with such properties may provide the highest target-to-nontarget ratio; therefore, they are most likely to be successful as imaging agents for Aβ plaques in the brain.

Experimental Section

Reagents used in the syntheses were purchased from Aldrich Chemical Co. or Fluka Chemical Co. and were used without further purification, unless otherwise indicated. Preparative thin-layer chromatography (PTLC) was performed on

silica gel plates with a fluorescent indicator that was visualized with light at 254 nm. ^{1}H NMR spectra were obtained on Bruker spectrometers (Bruker DPX 200 and AMX 500). Chemical shifts are reported as δ values and referenced to $CDCl_3$ (7.26 ppm for ^{1}H). Coupling constants are reported in hertz. Mass spectrometry was performed by the Mass Spectrometry Center, University of Pennsylvania. Elemental analysis was performed by Elemental Analysis Center, University of Pennsylvania. UV absorbance was measured on a Beckman DU640 spectrophotometer, and the fluorescence was recorded on a Perkin-Elmer LS55 luminescence spectrometer. Two high-pressure liquid chromatography (HPLC) systems were used to confirm the purity of some compounds listed in this section. In system A, a reversed-phase PRP-1 column (Hamilton, 250 × 4.6 mm) was eluted with CH_3CN/dimethyl glutarate buffer, 5 mM, pH 7.0 (9:1) at a flow rate of 1 mL/min. In system B, a normal-phase Partisil 10 column (Whatman, 250 × 4.6 mm) was eluted with hexanes/ethyl acetate (1:1) at a flow rate of 2.0 mL/min.

Absorbance (UV/vis) was measured with a spectrophotometer (Beckman DU 640). Fluorescence spectra were obtained with a luminescence spectrometer (Perkin-Elmer LS 55). Cells of 1 cm path length were used. Fluorescence quantum yields were obtained by comparing the area under the emission spectrum with that of a standard with a known quantum yield (quinine hydrochloride solution in 0.1 N H_2SO_4, $\phi = 0.55$).[24] Emission and excitation slits were set to 5 nm and the scan speed 500 nm/min. The temperature was ambient (24 °C). All of the compounds were dissolved in absolute ethanol, and concentrations between 10^{-5} and 10^{-6} M were used for absorbance measurements.

2-(4′-Nitrophenyl)imidazo[1,2-*a*]pyridine (12a). This compound was prepared by a previously reported method.[20] Yield 74%, mp 269−270 °C (lit.[20] mp 268−270 °C).

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.86 (dt, J = 1.1, 7.9 Hz, 1H), 7.22−7.28 (m, 1H), 7.68 (dd, J = 1.0, 9.2 Hz, 1H), 8.00 (s, 1H), 8.12 (d, J = 9.0 Hz, 2H), 8.17 (dt, J = 1.1, 6.7 Hz, 1H), 8.31 (d, J = 9.0 Hz, 2H).

6-Methyl-2-(4′-nitrophenyl)imidazo[1,2-*a*]pyridine (14a). This compound was prepared by a previously reported method.[20] Yield 85%, mp 245−247 °C (lit.[20] mp 235 °C).

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.35 (s, 3H), 7.10 (dd, J = 1.7, 9.3 Hz, 1H), 7.57 (d, J = 9.3 Hz, 1H), 7.91 (s, 1H), 7.94 (d, J = 1.7 Hz, 1H), 8.09 (d, J = 9.0 Hz, 2H), 8.29 (d, J = 9.0 Hz, 2H).

2-(4′-Aminophenyl)imidazo[1,2-*a*]pyridine (12b). This compound was prepared by a previously reported method.[21] Yield 86%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.72−6.78 (m, 1H), 6.75 (d, J = 8.5 Hz, 2H), 7.11−7.15 (m, 1H), 7.59 (dd, J = 0.7, 9.2 Hz, 1H), 7.74 (s, 1H), 7.76 (d, J = 8.5 Hz, 2H), 8.08−8.10 (m, 1H).

2-(4′-Methylaminophenyl)imidazo[1,2-*a*]pyridine (12c). A stirred suspension of **12b** (0.5 g, 2.39 mmol) in THF (5 mL) was treated dropwise with formic acetic anhydride (2 mL) at ambient temperature under nitrogen. After 30 min, the mixture was poured into 50 mL of ice-cold saturated aqueous Na_2CO_3. The precipitate was collected by vacuum filtration and dried in vacuo. The formamide was added in small portions to a stirred solution of $LiAlH_4$ (0.2 g, 5.3 mmol) in THF (10 mL), and the mixture was heated to reflux for 1 h. Upon cooling, the solution was cautiously quenched with aqueous KOH (1 N, 0.5 mL), diatomaceous earth was added, and the mixture was filtered. Solids were washed with acetone, and the combined filtrates were evaporated to give a light brown product (0.33 g, 62%). The purity of **12c** was evaluated on two HPLC systems and found to be greater than 95% (system A, retention time = 3.6 min; system B, retention time = 9.8 min).

^{1}H NMR (500 MHz, DMSO-d_6): δ 2.80 (s, 3H), 6.91 (d, J = 5.5 Hz, 2H), 7.47 7.50 (m, 1H), 7.87−7.96 (m, 4H), 8.64 (s, 1H), 8.86 (d, J = 6.5 Hz, 1H).

6-Methyl-2-(4′-methylaminophenyl)imidazo[1,2-*a*]pyridine (14c). The same procedure described above for the preparation of **12c** was performed. Starting from **14a**, a reduction reaction produced **14b**, and subsequent monomethylation yielded **14c** with a yield of 52%. The purity was evaluated on two HPLC systems and found to be greater than 95% (system A, retention time = 4.0 min; system B, retention time = 10.4 min).

^{1}H NMR (500 MHz, DMSO-d_6): δ 2.24 (s, 3H), 2.69 (d, J = 4.9 Hz, 3H), 5.75 (q, J = 4.9 Hz, 1H), 6.55 (d, J = 9.0 Hz, 2H), 7.00 (d, J = 9.0 Hz, 1H), 7.37 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 9.0 Hz, 2H), 8.00 (s, 1H), 8.22 (s, 1H).

2-(4′-Dimethylaminophenyl)imidazo[1,2-*a*]pyridine (13). To a mixture of **12c** (23 mg, 0.11 mmol) and $(CH_2O)_n$ (100 mg) in THF (5 mL) was added $NaBH_4$ (100 mg) in one portion at room temperature, followed by TFA (2 mL) dropwise. The resulting mixture was stirred at room temperature overnight and poured into an ice-cold NaOH solution (25%). The mixture was extracted with CH_2Cl_2, dried, filtered, and concentrated to give a crude product which was purified by PTLC (EtOAc/Hex = 1:1 as developing solvent) to give 15 mg of product **13** (58% yield). The purity was evaluated on two HPLC systems and found to be greater than 95% (system A, retention time = 4.5 min; system B, retention time = 6.1 min).

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.99 (s, 6H), 6.70 (t, J = 6.8 Hz, 1 H), 6.78 (d, J = 8.8 Hz, 2H), 7.10 (t, J = 6.8 Hz, 1H), 7.58 (d, J = 9.5 Hz, 1H), 7.71 (s, 1H), 7.83 (d, J = 8.7 Hz, 2H), 8.05 (d, J = 6.7 Hz, 1H).

2-(4′-Dimethylaminophenyl)-6-methylimidazo[1,2-*a*]pyridine (15). The same procedure for preparation of **13** was performed to prepare **15**, starting from **14c**. The purity was evaluated on two HPLC systems and found to be greater than 95% (system A, retention time = 5.2 min; system B, retention time = 5.4 min).

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.29 (s, 3H), 2.99 (s, 6H), 6.78 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 9.2 Hz, 1 H), 7.58 (d, J = 9.2 Hz, 1H), 7.64 (s, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.85 (s, 1H).

2-(4′-Dimethylaminophenyl)-6-iodoimidazo[1,2-*a*]pyridine, 16(IMPY). A mixture of 2-bromo-4′-dimethylaminoacetophenone **10b** (484 mg, 2 mmol) and 2-amino-5-iodopyridine **11e** (440 mg, 2 mmol) in EtOH (25 mL) was stirred under reflux for 2 h. $NaHCO_3$ (250 mg) was added after the mixture was cooled. The resulting mixture was stirred under reflux for 4 h. The mixture was cooled and filtered to give 348 mg of product, **16**(IMPY) (48% yield).

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.00 (s, 6H), 6.77 (d, J = 8.8 Hz, 2H), 7.27 (dd, J = 9.4, 1.5 Hz, 1H), 7.38 (d, J = 9.5 Hz, 1H), 7.66 (s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 8.32 (d, J = 0.7 Hz, 1H). Anal. **16**, ($C_{15}H_{14}IN_3$).

6-Bromo-2-(4′-dimethylaminophenyl)imidazo[1,2-*a*]pyridine (17). A mixture of 2-bromo-4′-dimethylaminoacetophenone **10b**[18] (968 mg, 4 mmol) and 2-amino-5-bromopyridine **11d** (692 mg, 4 mmol) in EtOH (25 mL) was stirred under reflux for 2 h. $NaHCO_3$ (500 mg) was added after the mixture was cooled. The resulting mixture was stirred under reflux for 4.5 h. The mixture was cooled and filtered to give 655 mg of product **17** (52% yield).

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.00 (s, 6H), 6.78 (d, J = 8.7 Hz, 2H), 7.17 (dd, J = 9.5, 1.7 Hz, 1H), 7.49 (d, J = 9.5 Hz, 1H), 7.69 (s, 1H), 7.80 (d, J = 0.7 Hz, 2H), 8.21 (dd, J = 1.7, 0.8 Hz, 1H). Anal. **17**, ($C_{15}H_{14}BrN_3$).

2-(4-Bromophenyl)-6-methylimidazo[1,2-*a*]pyridine (18). This compound was prepared by a procedure reported previously.[27]

2-(4-Bromophenyl)-6-(dimethylamino)imidazo[1,2-*a*]pyridine (19). The same procedure described for the preparation of **17** was performed to prepare **19**, starting from α-bromo-4-bromoacetophenone **10a** (1.5 g) and 2-amino-5-(dimethylamino)pyridine **11a** (0.74 g), to give **19** in 38% yield.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.88 (s, 6H), 7.05 (dd, J = 2.34, 9.81 Hz, 1H), 7.37 (d, J = 1.89 Hz, 1H), 7.45−7.55 (m, 3H), 7.73−7.81 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 41.53, 107.85, 108.64, 117.03, 119.67, 121.23, 127.18, 131.68, 133.20, 140.13, 142.13, 143.90. Anal. **19**, ($C_{15}H_{14}BrN_3$).

2-(4′-Dimethylaminophenyl)-3-iodoimidazo[1,2-*a*]pyridine (20). To a solution of **13** (20 mg, 0.08 mmol) in $CHCl_3$-

(10 mL) was added I_2 solution (0.5 mL, 1 M in $CHCl_3$) dropwise at room temperature. The mixture was stirred at room temperature for 1 h. $NaHSO_3$ solution (2 mL, 5%) was added, followed by KF (2 mL, 1 M in MeOH). The organic phase was separated, dried, filtered, and concentrated to give a crude product, which was purified by PTLC (Hex/EtOAc = 1:1 as developing solvent) to give 16 mg of product **20** (52%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.02 (s, 6H), 6.82 (d, J = 7.1 Hz, 2H), 6.88 (t, J = 6.8 Hz, 1H), 7.22 (t, J = 6.8 Hz, 1H), 7.60 (d, J = 9.0 Hz, 1H), 7.98 (d, J = 9.0 Hz, 2H), 8.20 (d, J = 6.8 Hz, 1H). Anal. **20**, ($C_{15}H_{14}I_2N_3$).

2-(4′-Dimethylaminophenyl)-6-(tributylstannyl)imidazo-[1,2-*a*]pyridine (21). To a solution of 6-bromo-2-(4′-dimethylaminophenyl)imidazo[1,2-*a*]pyridinee **17** (80 mg, 0.26 mmol) in 1,4-dioxane (10 mL) and triethylamine (2 mL) was added $(Bu_3Sn)_2$ (0.2 mL, neat), followed by $Pd(Ph_3P)_4$ (20 mg). The mixture was stirred at 90 °C overnight. Solvent was removed, and the residue was purified by PTLC (Hex/EtOAc = 1:1 as developing solvent) to give 23 mg of product **21** (17%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 7.2 Hz, 9H), 1.10 (t, J = 8.0 Hz, 6H), 1.33 (hex, J = 7.1 Hz, 6H), 1.54 (pen, J = 7.2 Hz, 6H), 3.00 (s, 6H), 6.78 (d, J = 8.9 Hz, 2H), 7.11 (d, J = 8.8 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.71 (s, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 0.8 Hz, 1H). HRMS: m/z calcd for $C_{27}H_{42}N_3Sn$ ($M^+ + H$) 528.2400, found 528.2402. Anal. **21**, ($C_{27}H_{41}N_3Sn{\cdot}2H_2O$).

2-(4′-Dimethylaminophenyl)-3,6-diiodoimidazo[1,2-*a*]-pyridine (22). The same procedure described for the preparation of **20** was performed, starting from **21** to give **22** in 32% yield.

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.02 (s, 6H), 6.82 (d, J = 7.1 Hz, 2H), 7.37 (d, J = 0.92 Hz, 2H), 7.98 (d, J = 7.0 Hz, 2H), 8.42 (d, J = 1.1 Hz, 1H). Anal. **22**, ($C_{15}H_{13}I_2N_3$).

Preparation of Radioiodinated Ligands: [^{125}I]16-(IMPY) and [^{125}I]7(TZDM). The radioiodinated compound, [^{125}I]**7**(TZDM), was prepared according to the method described previously.[7] The desired [^{125}I]**16**(IMPY) was prepared using iododestannylation reactions with tributyltin precursor **21**.[28] Hydrogen peroxide (50 μL, 3% w/v) was added to a mixture of 50 μL of the corresponding tributyltin precursor (1 μg/μL EtOH), 50 μL of 1 N HCl, and [^{125}I]NaI (1−5 mCi, purchased from NEN) in a sealed vial. The reaction was allowed to proceed for 10 min at room temperature and terminated by addition of 100 μL of saturated $NaHSO_3$. The reaction mixture was extracted with ethyl acetate (3 × 1 mL) after neutralization with saturated sodium bicarbonate solution. The combined extracts were evaporated to dryness. The residues were dissolved in 100 μL of EtOH and purified by HPLC using a reversed-phase column (PRP-1, 250 × 4.6 mm) with an isocratic solvent consisting of 90% acetonitrile/10% 3,3-dimethylglutaric acid (5 mM, pH 7.0) at a flow rate of 1.0 mL/min. The no-carrier-added products were evaporated to dryness and redissolved in 100% EtOH (1 μCi/μL), The final [^{125}I]**16**(IMPY), with a specific activity of 2200 Ci/mmol and >95% radiochemical purity, was stored at −20 °C up to 6 weeks for in vitro binding and autoradiography studies.

Binding Assays Using Aggregated Aβ40 Peptide in Solution. The solid form of peptide Aβ40 was purchased from Bachem (King of Prussia, PA). Aggregation of peptide was carried out by gently dissolving the peptide (0.5 mg/mL) in a buffer solution (pH 7.4) containing 10 mM sodium phosphate and 1 mM EDTA. The solutions were incubated at 37 °C for 36−42 h with gentle and constant shaking. Binding studies were carried out in 12- × 75-mm borosilicate glass tubes according to the procedure described previously.[7] For inhibition studies, 1 mL of the reaction mixture contained 40 μL of inhibitors (concentration range between 10^{-5} and 10^{-10} M, diluted in 10% EtOH), 50 μL of aggregated fibrils (10−50 nM in the final assay mixture), and 0.05 nM radiotracer in 40% EtOH. Ethanol is needed for this assay; without it, some of the "cold" ligands evaluated were not soluble. Nonspecific binding was defined in the presence of 2 μM thioflavin-T. Under the assay conditions, the specifically bound fraction was less than 15% of the total radioactivity. The mixture was incubated at room temperature for 3 h, and the bound and the free radioactivities were separated by vacuum filtration through Whatman GF/B filters using a Brandel M-24R cell harvester, followed by 2 × 3 mL washes with 10% ethanol at room temperature. Filters containing the bound I-125 ligand were counted in a γ counter (Packard 5000) with 70% counting efficiency. The results of inhibition experiments were subjected to nonlinear regression analysis using EBDA software,[29] and K_i values were calculated.

In Vitro Autoradiography and Fluorescent Staining of Brain Sections of a Tg 2576 (APPSW) Mouse. The brain of a 16-month-old Tg2576 mouse was removed and frozen in powdered dry ice.[30] After equilibration to −20 °C, consecutive 20-μm coronal sections were cut on a cryostat (Hacker Instruments, Fairfield, NJ), thaw-mounted on Fisher Superfrost Plus slides, and stored at −70 °C until use. The sections were thawed, labeled with 0.3 nM [^{125}I]**16**(IMPY) at room temperature for 1 h, and washed 2 × 3 min with saturated Li_2CO_3 in 40% EtOH, 2 min in 40% EtOH, and 30 s in H_2O. After drying, the labeled sections were exposed to Cronex MRF film for 72 h, along with a strip of I-125 standard (0.5 cm wide) made of polymer containing 156, 81, 39.3, 20, 9.85, and 4.98 nCi/mg of activity (Amersham, Buckinghamshire, England). The films were developed and digitized using a computer-based image analysis system (NIH image, version 1.61). The presence of radiolabeled amyloid deposits in the sections was confirmed in the same section labeled with thioflavin-S (TF-S). Fluorescence staining of the brain tissue sections with TF-S was achieved by the following steps: staining with TF-S for 3 min (0.0125% thioflavin-S in 40% EtOH/60% PBS). Sections are quickly differentiated in 50% EtOH/50% PBS for 3 min, PBS for 1 min, and H_2O for 5 min prior to imaging by fluorescent microscopy (BioRad 1024-ES and Nikon Eclipse E-600) using an FITC cube with an exitation filter of 350−390 nm and an emission filter of 530 ± 15 nm.

In Vivo Biodistribution in Normal Mice. While under ether anesthesia, male ICR mice (2−3 months old, average weight 20−30 g) received an injection directly into the tail vein of 0.15 mL of a 0.1% bovine serum albumin solution containing [^{125}I]**16** (5−10 μCi). The mice were sacrificed by cardiac excision at various time points postinjection. The organs of interest were removed and weighed, and the radioactivity was counted with an automatic γ counter (Packard 5000). The percentage dose per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material. Total activities of blood and muscle were calculated under the assumption that they were 7% and 40% of the total body weight, respectively.

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