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Synthesis of 5- and 6-substituted 2-(4-dimethylaminophenyl)-1,3-benzoxazoles and their in vitro and in vivo evaluation as imaging agents for amyloid plaque

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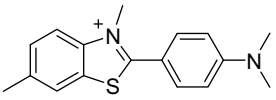
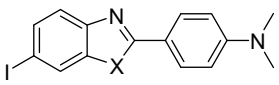
ABSTRACT

A series of novel 5- and 6-substituted 2-(4-dimethylaminophenyl)-1,3-benzoxazoles was synthesized and their potential as imaging probes for Alzheimer's Disease (AD)-related amyloid plaque was evaluated in vitro and in vivo. In vitro binding affinities for Aβ1–40 peptide of several of these compounds were in the low-nanomolar range . The lowest K_i of 9.3 nM was found for *N*-(2-(4-(dimethylamino)phenyl)-1,3-benzoxazol-5-yl)-4-iodobenzamide (**1e**). Its ^{123}I-radiolabeled form ([^{123}I]**1e**) was subsequently prepared by iododestannylation of the corresponding tributylstannyl precursor and evaluated in vivo in a baboon model using SPECT imaging. Contrary to our expectations, **1e** did not cross the blood–brain barrier (BBB) to any significant extent.

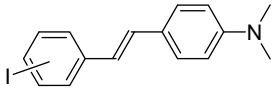
Alzheimer's Disease (AD) is the most common form of dementia in people aged 65 and older. Currently, several million patients suffer from AD in the US alone, and with the aging of the baby-boom generation, this number is expected to increase. AD is a neurodegenerative disease that gradually leads to severe memory loss. Abundant insoluble neurotoxic deposits of Aβ1–40 and Aβ1–42 peptides on neurons ('Aβ-plaque' or 'amyloid plaque'), resulting from cleavage of the amyloid precursor protein (APP) by specific proteases, are one of the characteristic pathological changes occurring in AD. Currently, a definite confirmation of AD is attained only by post-mortem histopathology of the brain. The ability to image amyloid plaque noninvasively in vivo would allow for early diagnosis of AD and evaluation of medication treatment progress in AD patients. Recently, it was reported that non-ionic analogues of Thioflavin T, an ionic imaging dye, penetrate the blood–brain barrier (BBB) and show high affinity for Aβ-plaque.[1] Radioiodinated varieties of these compounds have been used for single photon emission computed tomography (SPECT) imaging of amyloid plaque.[2–4]



Thioflavin T

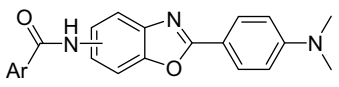


IBOX (X= O); TZDM (X= S)



I-Stilbene

A number of these ligands share a 4-(dimethylamino)phenyl fragment linked to another aromatic moiety, such as 5- or 6-substituted benzothiazole or benzoxazole.[5–8] Given the favorable Aβ-plaque binding properties shown in initial studies, we decided to further explore IBOX-related structures.[2] We reasoned that replacing the original iodo substituent with an amine functionality would allow for easy attachment of different groups via an amide bond. Here we report the synthesis and in vitro and in vivo evaluation of 5- and 6-substituted 2-(4-dimethylaminophenyl)-1,3-benzoxazoles (**1**) as potential imaging agents for amyloid plaque.



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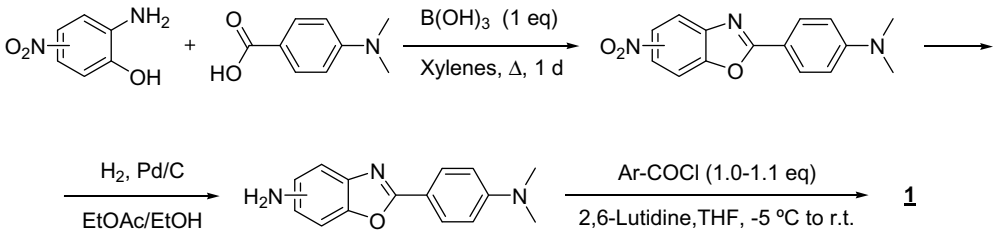


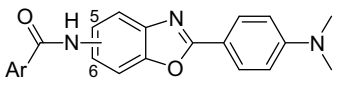
Scheme 1. Synthesis of the target substituted benzoxazoles.

Compounds of the general structure **1** were synthesized according to Scheme 1. The 1,3-benzoxazole nucleus was formed via a boronic acid-catalyzed condensation of the corresponding aminonitrophenols with 4-(dimethylamino)benzoic acid. Reduction of the nitro group to amino followed by condensation with the appropriate aromatic acid chloride gave the intended structures. Purity of the target compounds was confirmed by ^{1}H and ^{13}C NMR, high-resolution mass spectrometry, and elemental analysis (C, H, N) (see Table 1).

Compounds were then screened for binding to Aβ-plaque by ELISA.[6] K_i values in the low-nanomolar range were found for several compounds. The in vitro data showed that a benzamide substituent in position 5 of the benzoxazole nucleus resulted in higher Aβ-plaque binding affinity compared to the same substituent in position 6. Further substitution of the benzamide moiety with a small, nonpolar group resulted in only minor to moderate effects on K_i: substitution at positions 3 and 4 of the benzamide phenyl had the least effect on binding (cf. **1a**, **1e**, **1g**, and **1i**). Fortunately, **1e**, the compound that exhibited the lowest K_i value in the series studied, was also the ideal candidate for developing a ^{123}I-labeled SPECT imaging probe.

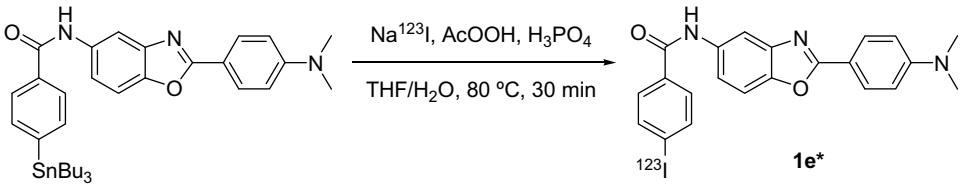
The radioiodinated [^{123}I]**1e** was prepared by reaction of the corresponding tributylstannyl precursor with electrophilic [^{123}I]iodine species generated *in situ* from sodium [^{123}I]iodide (Scheme 2). In a typical procedure, to a vial containing Na[^{123}I]I and NaOH were added: reaction solvent (50% THF); 0.8 M H_3PO_4 to adjust pH to 3; 50–100 μg of the radiolabeling precursor in 50 μL of THF; and peracetic acid so that its final concentration was at least 1%. The reaction mixture was heated at 80 °C for 30 min, quenched with 100 μL (100 μg) of $Na_2S_2O_5$/$NaHCO_3$ solution, and injected onto a C18 reverse-phase HPLC column eluted with a mixture of MeCN–H_2O–Et_3N (70:30:0.2, v/v/v). The fraction containing [^{123}I]**1e** was collected, diluted with water, and the resulting solution was passed through a C18 solid-phase extraction cartridge.

Table 1
Properties of the novel 5- and 6-substituted 2-(4-dimethylaminophenyl)-1,3-benzoxazoles



Compound	Ar	Position	Yield[a] (%)	Mp (°C)	K_i[b] (nM)	Clog$P$[c]
1a	Phenyl	5	58	251–254	12.0	4.92
1b	Phenyl	6	41	259–263	26.0	4.92
1c	3,4,5-Trimethoxyphenyl	5	47	224–226	109	4.28
1d	3,4,5-Trimethoxyphenyl	6	64	215–217	628	4.28
1e	4-Iodophenyl	5	34	274–279	9.3	6.11
1f	4-Iodophenyl	6	59	273–278	60.1	6.11
1g	*p*-Tolyl	5	42	255–258	13.2	5.42
1h	*p*-Tolyl	6	45	270–273	86.0	5.42
1i	*m*-Tolyl	5	24	193–195	13.4	5.42
1j	*m*-Tolyl	6	44	192–196	31.5	5.42
1k	*o*-Tolyl	5	38	176–178	18.9	5.08
1l	*o*-Tolyl	6	50	195–198	112	5.08
1m	3,4-(Methylenedioxy)phenyl	5	30	262–266	17.2	4.60
1n	3,4-(Methylenedioxy)phenyl	6	52	244–246	19.7	4.60
PIB[d]					4.3	3.99

[a] After purification, last step, not optimized.
[b] Against [^{3}H]BTA-1 as obtained in ELISA.[6]
[c] As calculated in ChemDraw Ultra 8.0 (CambridgeSoft, Cambridge, MA).
[d] Reference compound.[6]



Scheme 2. Synthesis of the putative [^{123}I]-labeled SPECT imaging probe [^{123}I]**1e**.

The product retained on the cartridge was eluted with 0.9 mL of 100% ethanol and filtered through a 0.22 μ membrane filter, then diluted with 9 mL of sterile normal saline. Radiolabeling yield was in the range of 60–70%, and the radiochemical purity of the final product exceeded 99%.

In vivo studies with [^{123}I]**1e** were carried out in ovariectomized female baboons (*Papio Anubis*). SPECT imaging was carried out as previously described by Staley et al.[9] under institutional animal care protocols complying with Federal regulations. In brief, a fasted (18–24 h) animal was immobilized using ketamine (10 mg/kg im), combined with glycopyrrolate (10 μg/kg im) 2 h prior to study. The animal was intubated and maintained on 2.5% isoflurane. Vital signs, including heart rate, respiration rate, oxygen saturation, and body temperature, were monitored every 15 min throughout the study. SPECT data were acquired with the nonhuman primate brain-dedicated multislice CERASPECT camera (Digital Scintigraphics, Waltham, MA, USA). Brain images (128 × 128 × 64 matrix; pixel size = 1.67 × 1.67 mm, slice, thickness = 1.67 mm, voxel volume = 4.66 mm^3) were acquired continuously at 159 keV for 1 h after injection of radiotracer (3.2 mCi in 8 mL). SPECT images were reconstructed using a ramp and a Butterworth filter (cut-off = 0.65 cm^{-1}, power factor = 10). Images showed low uptake in the brain and no distinct localization within brain regions.

These animal studies showed that, despite the observed in vitro binding of **1e**, radiolabeled [^{123}I]**1e** would not be a suitable radioligand for in vivo imaging of amyloid plaque. The inability of this compound to cross the BBB may be attributable to its excessively high lipophilicity (*C*Log *P* 6.11 compared to a range of 1.5–4 considered generally acceptable[10]). Modifications of the benzamide moiety of the general structure **1** aimed at lowering the lipophilicity might allow for penetrating the brain while maintaining favorable Aβ-plaque binding characteristics. Recent studies with modified amyloid ligands[11–13] provide encouragement to further explore this route.

Acknowledgments

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