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2-Arylimidazo[2,1-*b*]benzothiazoles: A new family of amyloid binding agents with potential for PET and SPECT imaging of Alzheimer's brain

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ABSTRACT

We designed and synthesized a small series of 2-aryl-imidazo[2,1-*b*]benzothiazole, representing a combination of motifs from the two most potent amyloid imaging agents, PIB and IMPY. The binding affinity of the new compounds ranged from 6 to 133 nM. Among the best compounds, **3b** (K_i = 6 nM) can be labeled with $^{11}CH_3$ for PET imaging whereas **3j** (K_i = 10.9 nM) can be labeled with ^{123}I for SPECT imaging.

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder affecting an estimated 35 million people worldwide.[1] AD is characterized by three structural changes: a diffuse neuron loss, intracellular deposit of neurofibrillary tangles (NFT) consisting mainly of hyper phosphorylated τ protein, and extracellular deposit of senile plaque consisting mainly of amyloid β peptide (Aβ). The etiology of the disease is largely unknown, but robust evidences (mainly genetic observations) implicate amyloid cascade as a central event in the development of the disease. Familial mutation within the presenilin 1 and 2 genes, which are critical for the catalytic activity of γ-secretase, cause an extremely aggressive and early form of AD.[2–4] Duplication of the amyloid precursor protein (APP) locus in extremely rare family mutation of chromosome 21 also induces early onset of AD,[5,6] The load of amyloid deposit in the brain correlates rather poorly with the cognitive deficit in AD[7,8] and a general consensus is emerging that soluble Aβ oligomers rather than plaque deposit[9–11] make up the toxic substance associated with cognitive impairment. Imaging amyloid deposits in living brain has stimulated much research in the past 20 years, with the goal of providing a reliable diagnostic for AD during the patient life (previously, only postmortem autopsy was available). Since this goal has been achieved with the discovery of [^{11}C]PIB[12], [^{123}I]IMPY[13], and other amyloid probes[14,15] such as [^{18}F]florbetaben and [^{18}F]FDDNP (Fig. 1), the new goal in AD imaging is its early diagnosis and better quantification for monitoring the amyloid burden in the brain.

In an attempt to develop more promising amyloid imaging agents, we synthesized a new series of 2-aryl-imidazo[2,1-*b*]benzothiazole derivatives and tested their binding to amyloid $A\beta_{1-40}$. This series was chosen since it combines key structural motifs shared by the most effective current agents, PIB and IMPY, possessing the benzothiazole part of PIB fused with the 2-arylimidazo part of IMPY (Fig. 1). We report here our initial results in the synthesis and in vitro evaluation of this new series of potential amyloid imaging agent.

The general synthesis of 2-aryl-imidazo[2,1-*b*]benzothiazole, outlined in Scheme 1, followed the classical[16,17] condensation of commercially available 2-aminobenzothiazole (**1**) with 2-bromoacetophenone (**2**) in the presence of sodium hydrogen carbonate, to provide, upon cooling and filtration, the expected compound **3** in good to excellent yield. 4′-Iodo-2-bromoacetophenone was prepared by simple bromination of 4-iodoacetophenone, while 4′-dimethylamino-2-bromoacetophenone was obtained in two steps following a previously published methodology.[18] The amino derivatives were obtained by reduction of the nitro analogues using tin chloride in good yield (a relatively large amount of EtOH is needed since both reagent and product have a relatively low solubility in EtOH). Selective monomethylation was achieved in one step and in excellent yield using the methodology developed by Barluenga et al.[19] All compounds were characterized by ^{1}H and ^{13}C NMR, HR-mass spectrometry, elemental analysis, and their purity was confirmed by HPLC and exceeds 96%.

All compounds were evaluated for their affinity to synthetic amyloid plaque ($A\beta_{1-40}$) in a competitive binding assay against [^{3}H]BTA-1 ([^{3}H]PIB) at a concentration of 1 nM; for comparison

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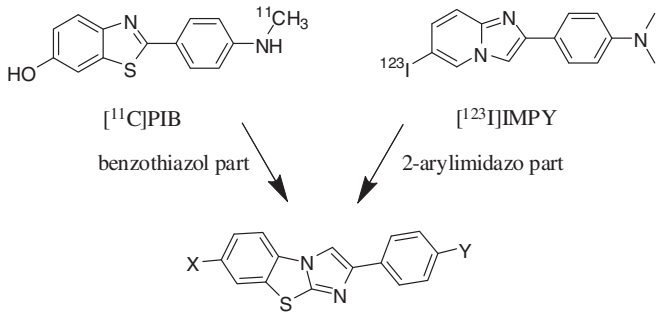


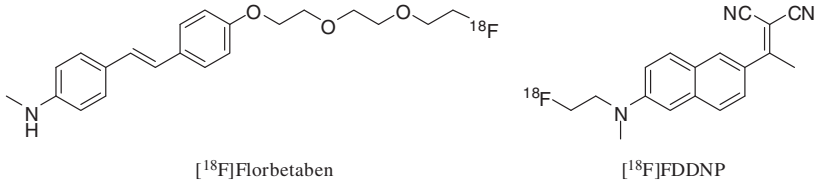
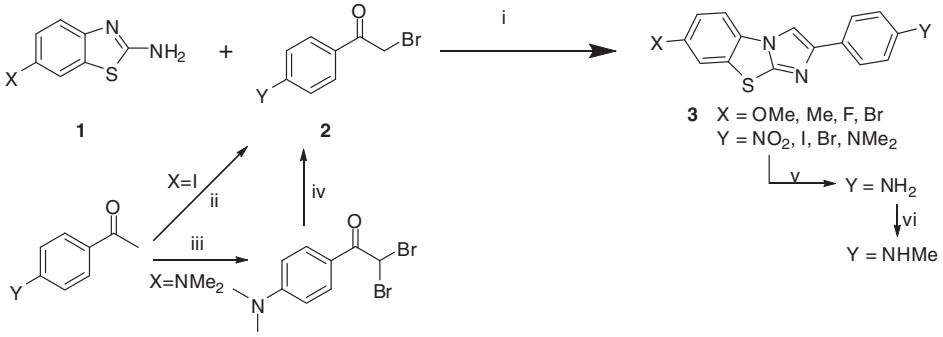


Figure 1.

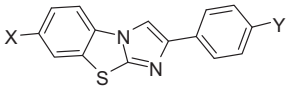


Scheme 1. Synthesis of 2-aryl-imidazo[2,1-*b*]benzothiazole. [a]Reagents and conditions: (i) EtOH, reflux 2 h, then $NaHCO_3$, reflux 4 h, 45–69%; (ii) Br_2, CH_2Cl_2, RT, 72%; (iii) H_2SO_4, Br_2, RT, 16 h, 97%; (iv) THF, $PO(OEt)_2$, Et_3N, RT, 6 h, 76%; (v) EtOH, $SnCl_2{\cdot}2H_2O$, reflux, 4 h, 89–65%; (vi) MeOH, MeONa, $(HCHO)_n$, reflux 2 h, then $NaBH_4$, reflux 2 h, 72–83%.

purpose PIB and IMPY were screened in the same assay. Inhibition constants (K_i) were calculated from the Cheng–Prusoff equation ($K_i = IC_{50}/(1+[L]/K_D)$) using IC_{50} values obtained from a 12-concentration dose–response curve (3 μM-0.01 nM serial dilution), using K_D = 10 nM for [^{3}H]PIB (obtained by saturation experiments). Results are expressed as the mean ± SD of at least three independent experiments with less than 20% in SD and are summarized in Table 1. As a general trend, the monomethyl aniline analogs had better affinity than their dimethyl or unsubstituted analogues (**3b** vs **3a**,**c**; **3e** vs **3d**, **f**; 3**h** vs **3i**); however, the substituent on the benzothiazole side of the molecule (substituent X) is clearly important, since the ranking between NH_2, NHMe, and NMe_2 was reversed between X = OMe and F, whereas almost equal binding affinities were observed with X = Br. Among the benzothiazole substituents, X = OMe led to the best affinity (**3b**, **j**, **n** K_i = 6.09, 10.9, 9.40 nM, respectively), whereas fluorine systematically showed much lower binding independently of phenylimidazo substituent Y (**3d**–**f**, **k**, X = F, Y = NH_2, NHMe, NMe_2, I). More surprisingly, good to excellent binding affinity values were observed for compounds lacking the anilino substitution (**3j**–**o** 9.4 nM ⩽ K_i ⩽ 41.9 nM). Of particular interest, compound **3j** (K_i = 10.9 nM) presents the advantage of potential dual labeling with either ^{11}C on the benzothiazole ring or ^{123}I on the phenylimidazo side of the molecule (substituent Y); however, the higher lipophilicity of **3j**, due to the absence of the aniline moiety, might result in increased non-specific binding and therefore limits its use as in vivo imaging agent. The most potent compound of this series was compound **3b**, exhibiting an affinity intermediate between PIB (K_i = 4.5 nM)[20] and IMPY (K_i = 15.0 nM)[21] and should be easily labeled with $^{11}CH_3$ at the *O*-methoxy or *N*-methyl position.

In conclusion, we developed a novel series of amyloid binding agents based on a 2-aryl-imidazo[2,1-*b*]benzothiazole scaffold, which can be viewed as a condensed mix between PIB and IMPY. Most of the compounds showed acceptable binding affinity for synthetic $A\beta_{1-40}$ fibril, ranging from 133 nM to 6.09 nM, and the best compound in the series (**3b**) can potentially be labeled with $^{11}CH_3$ at either the O or N position for in vivo evaluation. These results support the hypothesis that mix-condensed analogues of PIB-

Table 1
Amyloid binding of 2-aryl-imidazo[2,1-*b*]benzothiazole derivatives



Compound	X	Y	K_i (nM)	*c* Log *P* [a]
3a	OMe	NH_2	29.8 ± 2.1	3.58
3b	OMe	NHMe	6.09 ± 0.08	3.88
3c	OMe	NMe_2	58.6 ± 4.7	4.66
3d	F	NH_2	133 ± 21	3.86
3e	F	NHMe	38.1 ± 2.6	4.16
3f	F	NMe_2	42.9 ± 5.7	4.95
3g	Br	NH_2	28.8 ± 1.2	4.53
3h	Br	NHMe	34.5 ± 3.5	4.83
3i	Br	NMe_2	43.4 ± 5.7	5.62
3j	OMe	I	10.9 ± 0.18	5.74
3k	F	I	41.9 ± 5.2	6.02
3l	Br	I	21.1 ± 0.9	6.69
3m	Me	I	17.7 ± 1.9	6.35
3n	OMe	Br	9.40 ± 0.07	5.21
3o	Me	Br	26.0 ± 0.9	5.82
PIB	–––	–––	11.0 ± 0.2 4.5[b]	3.41
IMPY	–––	–––	9.0 ± 0.9 15.0[b]	4.64

Inhibition constants were measured on $A\beta_{1-40}$ against [^{3}H]BTA-1 at 1 nM, using K_D = 10 nM. Results are expressed as mean ± standard deviation ($n \geqslant 3$).
[a] *c* Log *P* values are calculated using ChemDraw Ultra 10.0.
[b] Literature values for the reference compounds PIB[20] and IMPY.[21]

IMPY could achieve similar potency to those of the individual compounds.

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