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Letters

Metabolically Stabilized Benzothiazoles for Imaging of Amyloid Plaques

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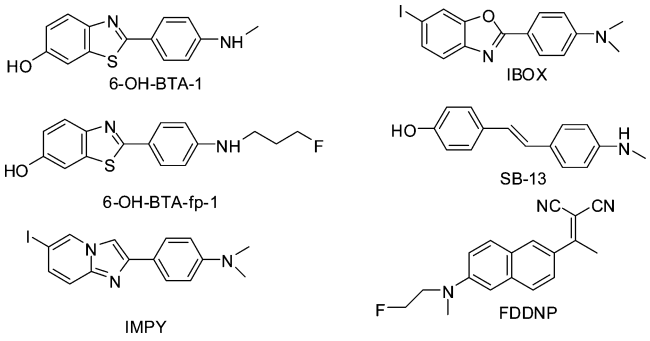
Abstract: Six new *N*-^{11}C-labeled aminophenylbenzothiazoles substituted with fluorine in different positions have been synthesized and evaluated as amyloid-β binding ligands. Our structure−property relationship studies show that the substitution pattern of the phenyl ring and the benzothiazole moiety has an influence on the metabolic stability, which in turn has an effect on the brain uptake kinetics. Two lead compounds have been identified with improved physicochemical characteristics for Aβ-plaque imaging in vivo.

The formation of β-amyloid (Aβ-plaques) is one of the earliest and most relevant pathological processes in the development of Alzheimer's disease (AD).[1] Therapeutic efforts have been focused on preventing or reversing Aβ-plaque deposition in the brain, and monitoring of the therapeutic efficacy would greatly benefit from methods for the in vivo detection and quantification of Aβ-deposits in the brain. Several radiolabeled compounds with high affinity and specificity for Aβ-aggregates have been developed. These compounds have the potential to bind and, by means of noninvasive imaging, to determine the localization and load of Aβ-plaques and thereby may allow the monitoring of the progression of this characteristic AD-feature non-invasively.[2−4]

Imaging agents for Aβ-plaques based on highly conjugated dyes such as congo red, chrysamine G, and thioflavin T, used in the fluorescent staining of plaques and tangles in postmortem AD brain sections, have been reported by several research groups. In vivo Aβ-plaque tracers for use in positron emission tomography (PET) based on 2-(4-aminophenyl)-benzothiazoles (BTAs), phenylimidazo [1,2-*a*] pyridines, styrenes, benzoxazoles. and a ^{18}F-labeled 6-dialkylamino-2-naphthyl-ethylidene-derivative [^{18}F]FDDNP (Chart 1) have been described. The first clinical trials in humans have already shown promising results.[5−10]

Chart 1. Amyloid-β Binding Compounds



The lead compound currently used in clinical trials, *N*-[^{11}C-methyl]-6-OH-BTA-1 ([^{11}C]PIB) has been shown to selectively enrich in frontotemporal and hippocampal areas of the brain in patients with AD compared with non-AD controls.[6,10] Despite the promising initial results, the regional uptake of [^{11}C]PIB is not correlating completely to the established regional pattern of plaque load as measured by post-mortem quantification.

As for other tracers designed for quantitative imaging in the central nervous system, the kinetic properties and rate of metabolism of Aβ-aimed radiopharmaceuticals is of outmost importance. A valid quantification of Aβ-aggregates in cerebral structures should ideally be done by determination of a metabolite-corrected plasma-input function. A rapid metabolism of the Aβ-tracer, therefore, not only limits the cerebral availability (signal strength), but also imposes difficulties to the determination of a valid input function. Until now, the influence of metabolic stability on the tracerkinetic properties of Aβ-imaging agents has barely been investigated.

N-[^{11}C-methyl]-6-OH-BTA-1 is known to be rapidly metabolized to the C6-sulfonated derivative.[11] In the present study, a new series of *N*-^{11}C-methylated-2-(4-aminophenyl)benzothiazoles were synthesized and their pharmacokinetic properties and

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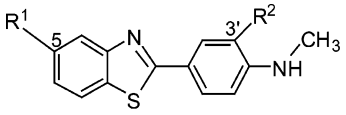
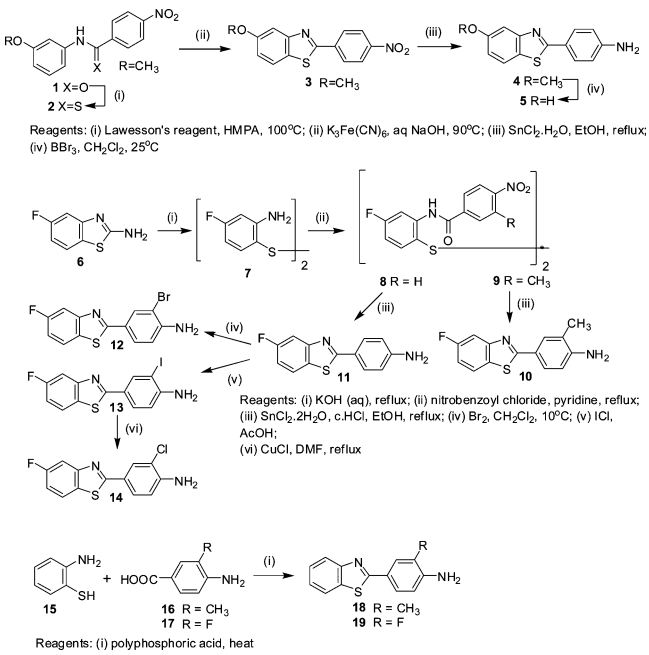
Table 1. Log $P_{oct/PBS}$, Brain Uptake Kinetics and Metabolic Stability in Mice, and Aβ(1−40) Binding Affinities for Benzothiazoles



entry	R^1	R^2	RCY[a] (%)	Log $P_{oct/PBS}$[b]	brain uptake 2 min[b]	brain uptake 2 min/30 min[b]	$T_{1/2}$ intact compound in plasma[a,c] (min)	K_i[d] (nM)
20	OH	H	28	1.35 ± 0.01	10.2 ± 0.9	10.6 ± 1.0	6.3	3.8
21	F	H	34	3.27 ± 0.05	6.1 ± 1.2	2.5 ± 0.8	6.8	12.4
22	F	Br	22	4.18 ± 0.07	4.1 ± 0.6	2.5 ± 0.9	7.2	1.3
23	H	CH_3	35	3.36 ± 0.04	5.6 ± 0.7	2.8 ± 0.6	7.5	4.4
24	H	F	17	3.05 ± 0.03	9.7 ± 1.3	3.1 ± 0.7	8.4	6.8
25	F	CH_3	37	2.93 ± 0.02	12.5 ± 2.2	6.3 ± 1.9	14.3	8.4
26	F	Cl	32	2.81 ± 0.02	19.3 ± 1.9	9.8 ± 1.1	22.1	3.4

[a] Average RCY at EOS, $n = 3-4$. [b] The results represent the means ± sd, $n = 4-5$. [c] Time postinjection at which 50% of the radioactivity in plasma corresponds to the intact compound. [d] Measured by competition binding studies to precipitates of synthetic Aβ (1−40) peptide.

Scheme 1. Syntheses of Benzothiazoles



Scheme 2. Example of Radiosynthesis of *N*-Methylated Benzothiazoles Using [^{11}C]CH_3OTf

rate of metabolic transformation were evaluated in mice. The primary intention was to evaluate whether BTAs substituted in the phenyl ring and the benzothiazole moiety, with variations to the substituent pattern on the aromatic rings, can lead to increased in vivo stability, yet providing a relevant affinity for the Aβ-target. Hence, six *N*-^{11}C-methylated-2-(4-aminophenyl)-benzothiazole compounds were selected, which combine structural diversity with the simplicity of ^{11}C-methylation for labeling.

The synthesis of precursor compounds (**5**, **10**, **11**, **12**, **14**, **18**, and **19**), based on previously reported methods,[12,13] is outlined in Scheme 1. A representative example of ^{11}C-methylation conditions to produce the secondary amine ([*N*-methyl-^{11}C]5-F,3′-Cl-BTA-1; [^{11}C]**26**) is shown in Scheme 2. Reaction of the primary amine precursors with [^{11}C]methyl triflate provided the new *N*-4′-^{11}C-methylated compounds [^{11}C]-**21**−**26** as well as [^{11}C]PIB ([^{11}C]**20**) in a specific activity >1000 Ci/mmol. The radiochemical yields (RCYs) at end-of-synthesis (EOS) were in the range 15−37% (Table 1). As can be seen from Table 1, among the halogens at the 3′-position, the RCYs obtained for ^{11}C-labeled compounds containing 3′-F and 3′-Br substituents ([^{11}C]**24** and [^{11}C]**22**, respectively, resulted in lower yields than the corresponding value obtained with 3′-Cl ([^{11}C]-**26**). The highest RCY was obtained with 3′-CH_3 ([^{11}C]**23** and-[^{11}C]**25**).

The compounds showed K_i values versus [^{3}H]PIB in the range of 1.3−12.4 nM on Aβ40 (Table 1), indicating that the alterations to the tested substitution pattern relative to [^{11}C]PIB do not affect a high affinity binding to Aβ40.

As the lipophilicity of drugs often correlates with their initial brain entry, the octanol−water partition coefficients (log $P_{oct/PBS}$) values for compound [^{11}C]**20**−**26** (Table 1) were measured. Log $P_{oct/PBS}$ was found to vary between 1.35 and 4.18, suggesting that all compounds should readily cross the blood−brain barrier. The brain uptake of compounds [^{11}C]**20**−**26** were subsequently measured in mice (wild type) at 2 and 30 min after intravenous (i.v.) injection (Table 1). Compared to the uptake of the reference [^{11}C]**20** in the brain of mice at 2 min (10.2 ± 0.9% ID/g), the uptake of the compounds [^{11}C]**21**−**26** were in the range of 4.1 ± 1.2 to 19.3 ± 1.9% ID/g (Table 1). The 2−30 min uptake ratios were in the range of 2.5 ± 0.9 to 9.8 ± 1.1 ([^{11}C]**20**: 10.6 ± 1.0).

To assess the impact of the BTA-substitution pattern on the rate of metabolism, the composition of radioactive species in plasma was analyzed following injections of compounds [^{11}C]-**20**−**26** into mice at 10 and 30 min p.i. by means of radio-HPLC. The extrapolated time interval to reach 50% of the intact compound in plasma within the series ranged from 6.8 to 22.1 min ([^{11}C]**20**: 6.3 min). All detected metabolites showed shorter retention times on the reverse-phase matrix. In separate experiments, the composition of radioactive species in brain tissue was analyzed using radio-HPLC following injections of compounds [^{11}C]**20**−**26**. Radiolabeled species were extracted from brain homogenates with ≥93% efficiency. No radioactive compounds except for the respective intact tracer were detectable (detection limit 2% of total analyzed activity).

Our two identified lead compounds [^{11}C]**25** and [^{11}C]**26** exhibited the highest brain uptake and highest stability toward metabolism. Interestingly, the compounds [^{11}C]**25** and [^{11}C]**26** showed a faster clearance than predicted from their relatively high Log $P_{oct/PBS}$ values, while having high brain uptake.

In this study, we focused primarily on establishing fundamental data on the effects of aromatic substitution pattern of BTAs on metabolic stability, lipophilicity, binding affinity for

Aβ40-fibrils, as well as its impact on the in vivo pharmacokinetics. We found that introduction of fluorine in the 5-position as well a formal substitution of 3′-H for F, Cl, Br, and CH_3 is compatible with pertaining a relevant affinity for Aβ. It was seen that 3′-Cl and -Br provided increased affinity relative to the H-analogues. An increased Aβ40 affinity of BTAs as a result of 3′-I substitution has been observed by other investigators.[14] Further, a fluorine substituent in the 5-position on the benzothiazole ring in combination with 3′-CH_3 or 3′-Cl significantly increased the metabolic stability relative to the reference [^{11}C]-PIB. Judged from the in vivo data obtained with the two leads, [^{11}C]**25** and [^{11}C]**26**, we demonstrated that the brain uptake kinetics of $N_{4'}$-^{11}C-methylated BTAs are controlled also by the metabolic stability of the compound.

We assessed brain uptake of the radiolabeled derivatives in wild type Balb-C mice without brain amyloid deposits. Thus, this study reflects brain entry and clearance from normal brain tissue. For imaging of Aβ in vivo, a high binding affinity, high brain entry, and low nonspecific binding in the brain will generally improve the information extracted from imaging protocols by increasing the signal-to-noise ratio. The requirement of rapid clearance from normal brain tissue is particularly important for PET ligands labeled with the short-lived ^{11}C ($t_{1/2}$ = 20.3 min). Previous studies[5,14] have emphasized the importance of providing a ^{11}C-labeled tracer of relatively low lipophilicity. Based on the 2 min/30 min uptake ratios found in this work (Table 1), it is indicated that a high lipophilicity (Log $P_{oct/PBS} \sim 3$) is not necessarily limiting. However, the optimum metabolic stability and overall brain uptake kinetics for BTA-based Aβ-imaging agents is yet to be established.

In summary, six novel N-^{11}C-labeled BTAs have been synthesized and screened for brain uptake and metabolic stability. It was found that the substitution pattern of the phenyl ring and the benzothiazole moiety has influence on the metabolic stability. The metabolic stability of the compounds in turn has an effect on the uptake of radioactivity in brain tissue. Two leads have been identified for which the increased metabolic stability has been found to lead to improved brain uptake kinetics. Work in transgenic animal models is in progress to further investigate whether the metabolically stabilized 5-,3′-substituted BTAs can provide improved specificity of the signal and facilitate a more precise quantification of the tracer kinetics.

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Supporting Information Available: General methods, experimental procedures, ^{13}C NMR shift values for reference compounds, determination of purity of test compounds, determination of K_i values, biodistribution in mice, and metabolite analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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