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EARLY DETECTION

Effects of Lipophilicity on the Affinity and Nonspecific Binding of Iodinated Benzothiazole Derivatives

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Abstract

A series of novel 2-aryl benzothiazole derivates substituted with iodine in different positions have been synthesized as amyloid-binding ligands. The affinity of these compounds for synthetic amyloid β (1–40) (Aβ[1–40]) fibrils was determined. Introduction of the iodo group in the position ortho to an amino group increased the binding affinity, whereas the iodination ortho to a hydroxyl group decreased the binding affinity. Selected compounds with high binding affinity and moderate lipophilicity (logP values, 1.65–3.90) were radiolabeled and evaluated in normal mice for brain uptake and clearance. Structure-activity relationship (SAR) studies showed a strong correlation between the lipophilicity of the iodinated compounds and the binding affinity as well as nonspecific binding. As the lipophilicity increased, the affinity for Aβ(1–40) fibrils improved; however, nonspecific binding in mouse brain reflected by low brain clearance also increased with increasing lipophilicity. These results provide important SAR information to guide the development of novel amyloid-binding agents and provide further insights into the molecular interaction between 2-aryl benzothiazole ligands and Aβ fibrils.

Index Entries: Radiopharmaceuticals; Alzheimer's disease; amyloid; benzothiazole.

Introduction

Aggregation and deposition of amyloid β (Aβ) peptides in the brain has been recognized as an early event in the pathogenesis of Alzheimer's disease (AD) (Selkoe, 2001). Within this pathological process, several targets have been identified for developing therapeutic agents aimed at the prevention or reversal of Aβ accumulation in the brain (Bard et al., 2000; Schenk et al., 2000; DeMattos et al., 2001; Olson et al., 2001). To evaluate the efficacy of amyloid therapies currently under development, efforts have been made to quantitate the level of amyloid deposition in vivo (Klunk et al., 1994; Selkoe, 2000). Several classes of compounds have been explored as amyloid-binding agents (Skovronsky et al., 2000; Styren et al., 2000; Wengenack et al., 2000; Klunk et al., 2002; Wang et al., 2002a). Our laboratory has developed lipophilic benzothiazole derivatives of thioflavin T that bind to amyloid deposits with high affinity and specificity (Klunk et al., 2001). Some lead compounds have been identified that exhibited both promising in vitro and in vivo properties. For example, 2-(4'-methylaminophenyl) benzothiazole (termed BTA-1) selectively bound to amyloid plaques in vivo, as demonstrated in a transgenic mouse model of amyloid deposition using multiphoton microscopy (Mathis et al., 2002). The binding of BTA-1 to post-

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mortem AD brain appeared specific for the Aβ deposits (Klunk et al., 2003). The clinical potential of these amyloid-binding ligands has been demonstrated with another lead compound, termed 6-OH-BTA-1 (2-[4'-methylaminophenyl])-6-hydroxybenzothiazole) (Engler et al., 2002). After radiolabeling with C-11, this compound readily entered the brain of human subjects and selectively bound to amyloid deposits in vivo in AD subjects. In contrast to normal healthy control subjects, the ligand was localized primarily to the amyloid-rich frontal and temporal cortices, as shown by positron emission tomography (PET) studies.

Based on these findings, we further extended the potential of 2-aryl benzothiazole amyloid-binding agents to the modality of single photon emission computed tomography (SPECT) by developing radioiodinated analogs. To date, several iodinated benzothiazole derivatives have been reported as amyloid-binding agents and demonstrated promising in vivo pharmacokinetics (Zhuang et al., 2001; Wang et al., 2002b). Despite the potential utility of these compounds in amyloid imaging, the key structural features necessary for high binding affinity and specificity are not well understood. This prompted us to synthesize a series of 2-aryl benzothiazole derivatives bearing iodo and other functional groups and compare the properties of amyloid binding affinity and in vivo brain entry and clearance.

We focused primarily on the effects of lipophilicity on the binding affinity for Aβ fibrils and pharmacokinetics in normal mouse brain. Lipophilicity, defined by the octanol-water partition coefficient (logP), is an important physicochemical parameter affecting the affinity of a molecule for lipophilic binding sites (Comer et al., 2001). Lipophilicity also has important effects on nonspecific binding in lipophilic environments, such as cell membranes. For small molecules, lipophilicity also plays a role in the movement across biological membranes such as the blood–brain barrier by passive diffusion. The optimal range of logP for brain entry is 0.9–2.5 (Dishino et al., 1983). Introduction of an iodo group typically increases the logP of aromatic compounds by 1.1 U (Hansch and Leo, 1979). Therefore, iodination can play a major role in the pharmacokinetics of central nervous system agents.

We describe the synthesis of these iodinated amyloid-binding ligands and the evaluation of their in vitro binding affinities, as well as in vivo brain uptake and clearance in normal control mice. Furthermore, structure-activity relationships (SARs) are established that correlate lipophilicity with affinity and nonspecific binding clearance from the brain. The SAR results help to identify the key structural features necessary for binding interaction with Aβ fibrils and may provide useful guidance in the further development of potential iodinated amyloid-binding agents for use with SPECT.

Materials and Methods

Study Design

Following the synthesis of each compound, the logP values, binding affinity, and brain entry and clearance were subsequently evaluated. Correlations between logP values and affinity or pharmacokinetic parameters were then performed.

Preparation of Ligands

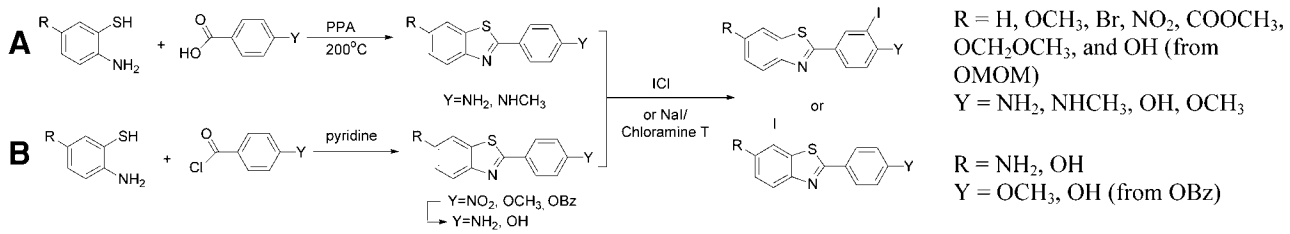
The synthesis of the iodinated compounds was achieved through direct electrophilic iodination of the corresponding 2-aryl benzothiazole derivatives, which were prepared through the approaches shown in Scheme 1. In approach A, amino or methylamino benzoic acid was used to couple with 5-substituted aminothiophenols in the presence of polyphosphoric acid at an elevated temperature. In approach B, *p*-nitro or *p*-methoxy benzoic acid chloride was used to react with 5-substituted aminothiophenols in the presence of pyridine at room temperature, followed by reduction of a nitro group to an amino group using tin chloride or by hydrolysis of the methoxy group to a hydroxyl group using boron tribromide.

Aromatic iodination was then performed under one of the following conditions: (1) iodochloride in acetic acid, or (2) sodium iodide in the presence of chloramine T. The structure of each compound was characterized by ^{1}H-NMR (300 MHz) and high-resolution mass spectroscopy.

Methods of Measurement

The lipophilicity in terms of logP value was determined based on a high-pressure liquid chromatograpy (HPLC) method (Mathis et al., in prep.). The logP values of the compounds were calculated based on the retention time on HPLC.

To determine the binding affinity for Aβ fibrils in terms of the inhibition constant (K_i), synthetic Aβ(1–40) (Bachem Bioscience) was allowed to aggregate into fibrils in phosphate buffered saline (pH 7.4) at room temperature. The Aβ(1–40) fibrils were then employed in the competitive binding assays using [^{3}H]BTA-1 as the radioligand. The in vitro binding studies were conducted at least in triplicate by the procedures described previously in detail (Klunk et



Scheme 1. Synthesis of benzothiazole derivatives and aromatic electrophilic iodination.

al., 2001; Mathis et al., 2002). For determination of K_i values, inhibition curves were fit (using the RS/1 statistical package, v. 6.1; Brooks Automation, Chelmsford, MA) to the following equation derived from the Hill equation: $F(x) = M(K_i)^H / ([L]^H + (K_i)^H)$, where M = maximal percent [^{3}H]BTA-1 bound (typical fit gave 100–104% for M), H is the Hill coefficient (typically 0.85–1.0), and L is the concentration of inhibitor compound.

For brain entry and clearance, selected compounds were radiolabeled with either ^{125}I or C-11 and introduced into wild-type, female Swiss-Webster mice through tail vein injection. At 2 and 30 min post-iv injection, mice were killed and radioactivity concentration, in terms of percent injected dose per gram tissue (%ID/g), was determined in whole brain. Radioactivity contents were assayed using a Gamma well-counter (Packard Instruments Model 5003, Meridan CT), and the counts were decay-corrected to the time of injection. Standards of the ^{125}I and C-11 injection solution were prepared and assayed using the Gamma well counter to determine the counting efficiency for calculating the %ID.

Data Analysis

The SAR studies were carried out by comparison of the binding affinity (K_i) and the 2/30 min ratio of %ID/g with the experimentally determined logP values.

Results

Introduction of an iodo group into a series of substituted 2-(4′-aminophenyl)benzothiazoles enhanced the binding affinity for Aβ(1–40) aggregates. The iodo group was introduced through direct aromatic eletrophilic iodination at the 3′ position ortho to the 4′-amino or methylamino group (Table 1). After iodination, the logP value increased by an average of 1.16 ± 0.07 U, consistent with the value predicted by Hansch substitutent constants (Hansch and Leo, 1979). All of these compounds bound to Aβ(1–40) aggregates. The K_i values were <55 nM for noniodinated derivatives and <16 nM for iodinated derivatives. Compared to that of 3′-H analogs, the K_i values of the 3′-I derivatives were on an average of 3.66 ± 2.61 times lower (i.e., higher affinity). The enhancement of the binding affinity was largest when an NH_2 group was present in the 4′ position, in which case an increase of fourfold or more was observed. Regardless of the substituents in the 6 position, the iodinated compounds with R' = CH_3 in the 4′ position exhibited an average of 2.83 ± 1.66-fold higher affinity than their analogs with R' = H.

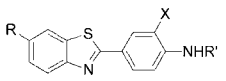
In contrast, binding affinity was decreased when the iodo group was introduced ortho to a hydroxyl group. This opposite effect of the iodo group was observed in two different series of substituted 2-(4′-hydroxyphenyl) benzothiazole derivatives. In one series the iodo group was introduced in the 3′ position (Table 2), and in the other series the 7 position was iodinated (Table 3). As shown in Table 2, the K_i values of 3′-iodinated derivatives were 3.70 ± 0.02-fold higher (i.e., lower affinity) than their noniodinated analogs (entries 13 and 14).

The logP values of these 4′-OH compounds bearing a hydroxyl in the 4′ position were increased by ~0.52 ± 0.14 U after iodination. This increase was 50% lower than the increase observed with 4′-amino analogs (entries 1–12).

Introduction of an iodo group at the 7 position produced similar effects in compounds having an NH2 or OH group in the 6 position (Table 3). Thus, iodination in the 7 position ortho to a NH_2 group in the 6 position increased the binding affinity (entries 16 and 17), whereas iodination in the 7 position ortho to an OH group in the 6 position decreased the binding affinity (entries 18 and 19). For 6-amino derivatives (entries 16 and 17), the logP values were increased by 1.1 U. For 6-hydroxyl derivatives (entries 18 and 19), the logP values were increased by 0.7 U after iodination.

Selected iodinated compounds (entries 1–4, X = I) were evaluated for brain uptake and clearance in normal mice (Table 4). After radiolabeling with either ^{125}I (entries 1 and 3) or C-11 (entries 2 and 4), these

Table 1
Binding Affinity (K_i) and Lipophilicity (logP) of Substituted 2-(4′-Aminophenyl) Benzothiazoles and 3′-Iodinated Analogs



			X=H		X=I		Comparison	
Entry	R	R'	K_i (nM)	logP	K_i (nM)	logP	Ki ratio	ΔlogP
1	H	H	36.8	1.98	8.32	3.17	4.42	1.19
2	H	CH_3	11.5	2.69	4.94	3.90	2.33	1.21
3	OH	H	45.6	0.66	11.1	1.65	4.11	0.99
4	OH	CH_3	4.3	1.23	3.22	2.35	1.34	1.12
5	NO_2	H	17.4	2.21	4.6	3.33	3.78	1.12
6	NO_2	CH_3	2.75	2.96	1	4.08	2.75	1.12
7	Br	H	7.22	2.87	0.67	4.11	10.78	1.24
8	Br	CH_3	1.69	3.64	1.6	4.86	1.06	1.22
9	CH_3O	H	7.00	1.87	4.4	3.08	1.59	1.21
10	CH_3O	CH_3	4.9	2.58	1.93	3.80	2.54	1.12
11	CH_3OCH_2O	H	53.6	1.86	15.1	3.03	3.55	1.17
12	CH_3OOC	H	17.9	2.07	3.34	3.29	5.36	1.22

$\Delta K_i = K_i$ (X = H)/K_i(X = I); ΔlogP = logP(X = I) – logP(X = H).

compounds were introduced into mice via tail vein injection and the radioactivity in the brain was assayed at 2- and 30-min time points. At 2 min post-iv injection, these compounds entered the mice brain fairly well with radioactivity concentration ranging from 4.40 to 9.08% ID/g. This concentration decreased to 3.4% ID/g or lower at 30 min with 2/30-min ratios ranging from 1.6 to 15.7. The 2/30-min ratios increased as the logP value decreased.

Discussion

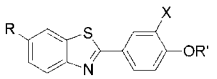
In this study we found that introduction of an iodo group in the position ortho to an amino group of a 2-(4′-aminophenyl)benzothiazole compound increased the binding affinity for Aβ(1–40) aggregates, whereas introduction of an iodo group in the position ortho to a hydroxyl group led to a decrease in the binding affinity. For the amino analogs, the effect of iodination and *N*-methylation both suggested that the benzothiazole binding site on Aβ(1–40) fibrils favors more lipophilic compounds. This same effect was observed with bromination (entries 7 and 8 vs 1 and 2). This trend was evidenced by the series of amyloid-binding ligands (entries 1–12, 16–17), in which the binding affinity increased as the lipophilicity of the ligands increased.

The opposite effect of iodination on the binding of benzothiazole compounds bearing hydroxyl groups (entries 13–15, 18–19) indicated that the binding interactions may also be affected by partial electron charges in the molecule. As iodine has an electronegativity of 2.5 in the Pauling scale relative to 2.1 for hydrogen, substitution of hydrogen with an iodo group in the 3′ position may enhance ionization of the OH group in the ortho position. For example, the pKa value of phenol is decreased from 10 to 8.51 by iodination in the ortho position (Massat et al., 1989), and deprotonation is increased under neutral conditions. The partial electron charge on the oxygen is thus increased, potentially hindering the binding interaction between the ligands and Aβ fibrils. In contrast, introduction of an iodo group ortho to an amino group decreases protonation in favor of the binding interaction.

For example, the pKa of aniline decreases from 4.6 to 2.55 by iodination in the ortho position (Arnett et al., 1970), indicating that the amino group would remain essentially uncharged under neutral binding conditions. The negative electronic effect of iodination ortho to a hydroxyl group on binding affinity appears to exceed the lipophilicity effect mentioned above as evidenced by the fact that iodinated hydroxyl compounds exhibited a binding affinity fourfold lower than the corresponding noniodinated analogs (Tables 2 and 3).

Binding affinity and brain entry, as well as specific binding relative to nonspecific binding, are impor-

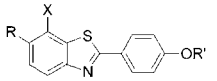
Table 2
Binding Affinity (K_i) and Lipophilicity (logP) of Substituted 2-(4′-Hydroxylphenyl) Benzothiazole Derivatives and Analogs Bearing the Iodo Group in 3′ Position



			X=H		X=I		Comparison	
Entry	R	R’	K_i (nM)	logP	K_i (nM)	logP	Ki ratio	ΔlogP
13	H	H	5.68	1.86	19.1	2.22	0.30	0.36
14	OH	H	16.8	0.39	71.2	1.09	0.24	0.7
15	OCH_3	H	4.2	1.8	15.8	2.31	0.27	0.51

ΔKi = K_i(X = H)/K_i(X = I); ΔlogP = logP(X = I) – logP (X = H).

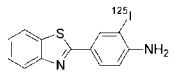
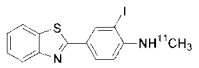
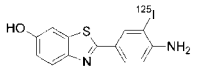
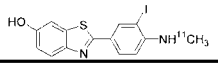
Table 3
Binding Affinity (K_i) and Lipophilicity (logP) of 2-Aryl Benzothiazole Derivatives Bearing an Iodo Group in the 7 Position and a 6 Position NH_2 or OH Group



			X=H		X=I		Comparison	
Entry	R	R’	K_i (nM)	logP	K_i (nM)	logP	Ki ratio	ΔlogP
16	NH_2	H	52.8	0.7	26.3	1.6	2.00	0.9
17	NH_2	CH_3	6.9	1.76	3.6	2.95	1.92	1.19
18	OH	H	16.8	0.39	34.5	1.01	0.49	0.62
19	OH	CH_3	6.3	1.75	7.1	2.49	0.89	0.74

ΔKi = K_i(X = H)/K_i(X = I); ΔlogP = logP(X = I) – logP (X = H).

Table 4
Brain Entry and Clearance of Selected Iodinated Compounds Radiolabeled with Either 125I or C-11

Entry	Structures	K_i (nM)	logP	2 min (%ID/g)	30 min (%ID/g)	2:30 min ratio
1		8.32	3.17	9.08	3.4	2.7
2		4.94	3.90	4.40	2.68	1.6
3		11.1	1.65	5.64	0.36	15.7
4		3.22	2.35	7.76	2.66	2.91

tant factors in the development of in vivo amyloid ligands. High binding affinity, high brain entry, and low nonspecific binding in the brain will generally improve the quality of tomographic studies by increasing the signal-to-noise ratio. As shown in Table 4, these radiolabeled compounds entered the brain well at the early time point with radioactivity concentration reaching >4%ID/g (a value typical of useful tracers for PET or SPECT). The above studies indicate that lipophilicity both enhanced binding affinity of compounds for Aβ aggregates and simultaneously decreased the rate of nonspecific binding clearance from the brain, as measured by the ratio of brain entry at 2 and 30 min. Our results indicated that 2-(3′-iodo-4′-aminophenyl)-6-hydroxybenzothiazole (entry 3, X = I) and 2-(3′-iodo-4′-methylaminophenyl)-6-hydroxybenzothiazole (entry 4, X = I) displayed rapid nonspecific binding clearance from the brain

and relatively high binding affinity. Further evaluation of these lead compounds is in progress.

Acknowledgments

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