THIOFLAVIN DERIVATIVES FOR USE IN THE ANTEMORTEM DIAGNOSIS OF ALZHEIMER'S DISEASE AND IN VIVO IMAGING AND PREVENTION OF AMYLOID DEPOSITION

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ABSTRACT

This invention relates to novel thioflavin derivatives, methods of using the derivatives in, for example, in vivo imaging of patients having neuritic plaques, pharmaceutical compositions comprising the thioflavin derivatives and method of synthesizing the compounds. The compounds find particular use in the diagnosis and treatment of patients having diseases where accumulation of neuritic plaques are prevalent. The disease states or maladies include but are not limited to Alzheimer's Disease, familial Alzheimer's Disease, Down's Syndrome and homozygotes for the apolipoprotein E4 allele.
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CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 12/046,070, file on Mar. 11, 2008, which is a continuation of U.S. application Ser. No. 10/859,600 filed Jun. 3, 2004, which is a continuation of U.S. application Ser. No. 09/935,767 filed Aug. 24, 2001 that claims priority from U.S. Provisional Application No. 60/227,601, filed Aug. 24, 2000, all of which applications are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention relates to the identification of compounds that are suitable for imaging amyloid deposits in living patients. More specifically, the present invention relates to a method of imaging amyloid deposits in brain in vivo to allow antemortem diagnosis of Alzheimer’s Disease. The present invention also relates to therapeutic uses for such compounds.

BACKGROUND OF THE INVENTION

[0003] Alzheimer’s Disease (“AD”) is a neurodegenerative illness characterized by memory loss and other cognitive deficits. McKhann et al., Neurology 34: 939 (1984). It is the most common cause of dementia in the United States. AD can strike persons as young as 40-50 years of age, yet, because the presence of the disease is difficult to determine without dangerous brain biopsy, the time of onset is unknown. The prevalence of AD increases with age, with estimates of the affected population reaching as high as 40-50% by ages 85-90. Evans et al., JAMA 262: 2551 (1989); Katzman, Neurology 43: 13 (1993).

[0004] In practice, AD is definitively diagnosed through examination of brain tissue, usually at autopsy. Khachaturian, Arch. Neurol. 42: 1097 (1985); McKhann et al., Neurology 34: 939 (1984). Neuropathologically, this disease is characterized by the presence of neuritic plaques (NP), neurofibrillary tangles (NFT), and neuronal loss, along with a variety of other findings. Mann, Mech. Ageing Dev. 31: 213 (1985). Post-mortem slices of brain tissue of victims of Alzheimer’s disease exhibit the presence of amyloid in the form of proteinaceous extracellular cores of the neuritic plaques that are characteristic of AD.


[0006] The initial deposition of Aβ probably occurs long before clinical symptoms are noticeable. The currently recommended “minimum microscopic criteria” for the diagnosis of AD is based on the number of neuritic plaques found in brain. Khachaturian, Arch. Neurol., supra (1985). Unfortunately, assessment of neuritic plaque counts must be delayed until after death.


[0008] Thus far, diagnosis of AD has been achieved mostly through clinical criteria evaluation, brain biopsies and post-mortem tissue studies. Research efforts to develop methods for diagnosing Alzheimer’s disease in vivo include (1) genetic testing, (2) immunossay methods and (3) imaging techniques.


[0011] Immunossay methods have been developed for detecting the presence of neurochemical markers in AD patients and to detect an AD related amyloid protein in cerebral spinal fluid. Warrer, Anal. Chem. 59: 1203A (1987); World Patent No. 92/17152 by Potter; Glenner et al., U.S. Pat. No. 4,666,829. These methods for diagnosing AD have not
been proven to detect AD in all patients, particularly at early stages of the disease and are relatively invasive, requiring a spinal tap. Also, attempts have been made to develop monoclonal antibodies as probes for imaging of Aβ. Majocha et al., J. Nucl. Med., 33: 2184 (1992); Majocha et al., WO 89/06242 and Majocha et al., U.S. Pat. No. 5,231,000. The major disadvantage of antibody probes is the difficulty in getting these large molecules across the blood-brain barrier. Using antibodies for in vivo diagnosis of AD would require marked absorb filter in the blood-brain barrier in order to gain access into the brain. There is no convincing functional evidence that abnormalities in the blood-brain barrier reliably exist in AD. Kalaria, Cerebrovascular & Brain Metabolism Reviews 4: 226 (1992).

[0012] Radiolabeled Aβ peptide has been used to label diffuse, compact and neuritic type plaques in sections of AD brain. See Maggio et al., WO 93/04194. However, these peptides share all of the disadvantages of antibodies. Specifically, peptides do not normally cross the blood-brain barrier in amounts necessary for imaging and because these probes react with diffuse plaques, they may not be specific for AD.

[0013] The inability to assess amyloid deposition in AD until after death impedes the study of this devastating illness. A method of quantifying amyloid deposition before death is needed both as a diagnostic tool in mild or clinically confusing cases as well as in monitoring the effectiveness of therapies targeted at preventing Aβ deposition. Therefore, it remains of utmost importance to develop a safe and specific method for diagnosing AD before death by imaging amyloid in brain parenchyma in vivo. Even though various attempts have been made to diagnose AD in vivo, currently, there are no antemortem probes for brain amyloid. No method has utilized a high affinity probe for amyloid that has low toxicity, can cross the blood-brain barrier, and binds more effectively to AD brain than to normal brain in order to identify AD amyloid deposits in brain before a patient’s death. Thus, no in vivo method for AD diagnosis has been demonstrated to meet these criteria.


[0015] It is known in the art that certain azo dyes, such as Congo red, may be carcinogenic. Morgan et al. Environmental Health Perspectives, 102 (supp.) 2: 63-78, (1994). This potential carcinogenicity appears to be based largely on the fact that azo dyes are extensively metabolized to the free parent amine by intestinal bacteria. Cermiglia et al., Biochem. Biophys. Res. Com., 107: 1224-1229, (1982). In the case of benzidine dyes (and many other substituted benzidines), it is the free amine which is the carcinogen. These facts have little implications for amyloid imaging studies in which an extremely minute amount of the high specific activity radio-labelled dye would be directly injected into the blood stream. In this case, the amount administered would be negligible and the dye would by-pass the intestinal bacteria.

[0016] In the case of therapeutic usage, these facts have critical importance. Release of a known carcinogen from a therapeutic compound is unacceptable. A second problem with diuzo dye metabolism is that much of the administered drug is metabolized by intestinal bacteria prior to absorption. This lowered bioavailability remains a disadvantage even if the metabolites released are innocuous.

[0017] Thioflavin T is a basic dye first described as a selective amyloid dye in 1959 by Vassar and Culling (Arch. Pathol. 68: 487 (1959)). Schwartz et al. (Zbl. Path. 106: 320 (1964)) first demonstrated the use of Thioflavin S, an acidic dye, as an amyloid dye in 1964. The properties of both Thioflavin T and Thioflavin S have since been studied in detail. Kelenyi J. Histochem. Cytochem. 15: 172 (1967); Burns et al. J. Path. Bact. 94: 337 (1967); Guntern et al. Experientia 48: 8 (1992); LeVince Meth. Enzymol. 309: 274 (1999). Thioflavin S is commonly used in the post-mortem study of amyloid deposition in AD brain where it has been shown to be one of the most sensitive techniques for demonstrating senile plaques. Vallet et al. Acta Neuropathol. 83: 170 (1992). Thioflavin T has been frequently used as a reagent to study the aggregation of soluble amyloid proteins into beta-sheet fibrils. LeVince Prot. Sci. 2: 404 (1993). Quaternary amine derivatives related to Thioflavin T have been proposed as amyloid imaging agents, although no evidence of brain uptake of these agents has been presented. Caprath et al. U.S. Pat. No. 6,001,331.

[0018] Thus, a need exists for amyloid binding compounds which enter the brain and bind selectively to amyloid.

[0019] A further need exists for amyloid binding compounds that are non-toxic and bioavailable and, consequently, can be used in therapeutics.

**SUMMARY OF THE INVENTION**

[0020] It is therefore one embodiment of the present invention to provide compounds which allow for a safe and specific method for diagnosing AD before death by in vivo imaging of amyloid in brain parenchyma.

[0021] It is another embodiment of the present invention to provide an approach for identifying AD amyloid deposits in brain before a patient’s death, using a high-affinity probe for amyloid which has low toxicity, can cross the blood-brain barrier, and can distinguish AD brain from normal brain.

[0022] In accomplishing these and other embodiments of the invention, there is provided, in accordance with one aspect of the invention, an amyloid binding compound having one of structures A-E:
wherein Z is S, NR', O or C(R')₂, in which case the correct tautomeric form of the heterocyclic ring becomes an indole in which R' is H or a lower alkyl group.

wherein Y is NR'R₂, OR₂, or SR₂; wherein the nitrogen of is not a quaternary amine; or an amyloid binding compound having one of structures F-J or a water soluble, non-toxic salt thereof:
wherein each Q is independently selected from one of the following structures:

![Structure Image]

wherein n = 0, 1, 2, 3 or 4,

wherein Y is NR'\(_1\)R'\(_2\), OR'\(_2\), or SR'\(_2\); wherein the nitrogen of

![Structure Image]

is not a quaternary amine; wherein each R'\(_3\) and R'\(_4\) independently is selected from the group consisting of H, a lower alkyl group, (CH\(_2\))\(_n\)OR' (wherein n = 1, 2, or 3), CF\(_3\), CH\(_2\)—CH\(_2\)X, CH\(_3\)—CH\(_2\)—CH\(_2\)X (wherein X = F, Cl, Br or I), (C—O)—R'\(_2\), R'\(_3\), and (CH\(_2\))\(_n\)R'\(_3\) (wherein n = 1, 2, 3, 4, and R'\(_3\) represents an unsubstituted or substituted phenyl group with the phenyl substituents being chosen from any of the non-phenyl substituents defined below for R'\(_3\)—R'\(_4\) and R' is H or a lower alkyl group); and

wherein each R'\(_3\)—R'\(_4\) independently are selected from the group consisting of H, F, Cl, Br, I, a lower alkyl group, (CH\(_2\))\(_n\)OR' (wherein n = 1, 2, or 3), CF\(_3\), CH\(_2\)—CH\(_2\)X, CH\(_2\)—CH\(_2\)—CH\(_2\)X, O—CH\(_2\)—CH\(_2\)X, CH\(_2\)—CH\(_2\)—CH\(_2\)X, O—CH\(_2\)—CH\(_2\)—CH\(_2\)X (wherein X = F, Cl, Br or I), CN, (C—O)—R'\(_2\), N(R'\(_2\))\(_2\), NO\(_2\), (C—O)N(R'\(_2\))\(_2\), O(CO)R'\(_2\), OR'\(_2\), SR'\(_2\), COOR'\(_2\), R'\(_3\), CR'\(_2\)—CR'\(_3\)—CR'\(_2\) (wherein R'\(_3\) represents an unsubstituted or substituted phenyl group with the phenyl substituents being chosen from any of the non-phenyl substituents defined for R'\(_3\)—R'\(_4\) and wherein R' is H or a lower alkyl group), a tri-alkyl tin and a chelating group (with or without a chelated metal group) of the form W—L or V—W—L, wherein V is selected from the group consisting of —COO—, —CO—, —CH\(_2\)O— and —CH\(_2\)NH—; W is —(CH\(_2\))\(_n\) where n = 0, 1, 2, 3, 4, or 5; and L is:

![Structure Image]
wherein \( M \) is selected from the group consisting of \( \text{Tc} \) and \( \text{Re} \); or wherein each \( R^1 \) and \( R^2 \) is a chelating group (with or without a chelated metal group) of the form \( W-L \), wherein \( W \) is \(-(\text{CH}_2)_n\) where \( n = 2, 3, 4, \) or \( 5 \); and \( L \) is:
and wherein $R'$ independently is selected from one of:

- $\text{CH}_3$
- $\text{COOH}$
- $\text{CONHCH}_2$
- $\text{SH}$
- $\text{HS}$
- $\text{OH}$

and wherein $R_{15}$ independently is selected from one of:

- $\text{H}$
- $\text{COOH}$
- $\text{CONHCH}_2$
- $\text{CH}_3$
- $\text{HS}$
- $\text{HO}$

or an amyloid binding, chelating compound (with or without a chelated metal group) or a water soluble, non-toxic salt thereof of the form:

and $R_{10}$ is

[0023]

wherein $Q$ is independently selected from one of the following structures:

- $\text{HO}$
- $\text{Z}$
- $\text{Q}$
wherein \( n = 0, 1, 2, 3 \) or \( 4 \), \( R_{17} \rightarrow R_{18} \rightarrow R_{17} \rightarrow R_{18} \rightarrow \) \( R_{19} \rightarrow R_{19} \) \( \\text{or} \) \( \rightarrow R_{20} \rightarrow R_{21} \rightarrow R_{19} \rightarrow R_{19} \rightarrow \) \( \rightarrow R_{19} \rightarrow R_{19} \rightarrow R_{20} \rightarrow \) wherein \( Z \) is \( S, NR', O, \) or \( C(R')_2 \) in which \( R' \) is \( H \) or a lower alkyl group; wherein \( U \) is \( N \) or \( CR' \); wherein \( Y \) is \( NR', OR^{25} \) or \( SR^{26} \); wherein each \( R^{17} \rightarrow R^{24} \) independently is selected from the group consisting of \( H, F, Cl, Br, I, \) a lower alkyl group, \((CH_2)_nOR'(\text{wherein} \ n = 1, 2, \text{or} 3)\), \( CF_3, \ CH_2\rightarrow CH_X, \ O\rightarrow CH_2\rightarrow CH_X, \ CH_2\rightarrow CH_2\rightarrow CH_X, \ O\rightarrow CH_2\rightarrow CH_2\rightarrow CH_X \) wherein \( X = F, \ Cl, Br \) or \( I \), \( CN, (C=O)\rightarrow R; N(R')_2, NO_2, \ (C=O)(N(R'))_2, O(CO)R', OR', SR', COOR', R_{phen}, CR'=CR', R_{poly} \) and \( CR'=CR', R_{phen} \) wherein \( R_{phen} \) represents an unsubstituted or substituted phenyl group with the phenyl substituents being chosen from any of the non-phenyl substituents defined for \( R^{17} \rightarrow R^{24} \) and wherein \( R' \) is \( H \) or a lower alkyl group).

[0024] In a preferred embodiment, at least one of the substituents \( R^{17} \rightarrow R^{18} \) of the structures \( A-E \) or \( F-J \) is selected from the group consisting of \( ^{131I}, ^{123I}, ^{75Br}, ^{18F}, \ CH_2, \ CH_2\rightarrow \ X, \ O\rightarrow CH_2\rightarrow CH_2\rightarrow X, \ CH_2\rightarrow CH_1\rightarrow CH_1\rightarrow X, \ O\rightarrow CH_2\rightarrow CH_2\rightarrow CH_1\rightarrow X \) (wherein \( X = ^{125I}, ^{123I}, ^{75Br}, ^{18F}, ^{18F}, ^{123I} \), a carbon-containing substituent as specified above wherein at least one carbon is \(^{12}C \) or \(^{13}C \) and a chelating group (with chelated metal group) of the form \( W-L^* \) or \( V-W-L^* \), wherein \( V \) is selected from the group consisting of \( \rightarrow COO, \rightarrow CO, \rightarrow CH_2O \) and \( \rightarrow CH_3NH \); \( W \) is \( \rightarrow (CH_2)_n \) wherein \( n = 0, 1, 2, 3, 4 \), or \( 5 \) and \( L^* \) is:

wherein \( M^* \) is \(^{99mTc} \); and a chelating group (with chelated metal group) of the form \( W-L^* \) or \( V-W-L^* \), wherein \( V \) is selected from the group consisting of \( \rightarrow COO, \rightarrow CO, \rightarrow CH_2O \) and \( \rightarrow CH_3NH \); \( W \) is \( \rightarrow (CH_2)_n \) wherein \( n = 0, 1, 2, 3, 4 \), or \( 5 \) and \( L^* \) is:
wherein $R'^{16}$ is

and $R'^{16}$ is

wherein $Q$ is independently selected from one of the following structures:

wherein $Z$ is S, NR', O, or C(R')$_2$ in which R' is H or a lower alkyl group;
wherein U is N or CR';
wherein Y is NR'$_2$, OR', or SR'$_2$;

wherein each $R'^{17}$-$R'^{24}$ independently is selected from the group consisting of H, F, Cl, Br, I, a lower alkyl group, (CH$_3$)$_2$OR' (wherein n=1, 2, or 3), CF$_3$, CH$_2$-$CH_2$X, O-CH$_2$-$CH_2$X, CH$_2$-$CH_2$-CH$_2$X, O-CH$_2$-$CH_2$-$CH_2$X (wherein X=F, Cl, Br or I), CN, (C(O)=)$-$R', N(R')$_2$, NO$_X$, (C(O)=O)(R')$_2$, O(CO)R', OR', SR', COOR', R$_p$/R$_n$, CR'$^2$-CR'$^2$-$R_p$ and CR'$^2$-$R_p$-CR'$^2$-$R_p$ (wherein R$_{p/n}$ represents an unsubstituted or substituted phenyl group with the phenyl substituents being chosen from any of the non-phenyl substituents defined for $R'^{17}$-$R'^{20}$ and wherein R' is H or a lower alkyl group).

In another preferred embodiment, the thioflavin compounds are defined where $Z$=S, $Y$=N, $R'^{16}$=H; and further wherein when the amyloid binding compound of the present invention is structure A or E, then $R'^{2}$ is selected from the group consisting of a lower alkyl group, (CH$_3$)$_2$OR' (wherein n=1, 2, or 3), CF$_3$, CH$_2$-$CH_2$X, CH$_2$-$CH_2$-$CH_2$X (wherein X=F, Cl, Br or I), (C(O)=)$-$R', R$_{p/n}$, and (CH$_2$)$_n$R$_{p/n}$ wherein n=1, 2, 3, or 4;

wherein when the amyloid binding compound of the present invention is structure B, then $R'^{2}$ is selected from the group consisting of (CH$_3$)$_2$OR' (wherein n=1, 2, or 3), CF$_3$, CH$_2$-$CH_2$X, CH$_2$-$CH_2$-$CH_2$X (wherein X=F, Cl, Br or I), (C(O)=)$-$H, R$_{p/n}$, and (CH$_2$)$_n$R$_{p/n}$ wherein n=1, 2, 3, or 4;

wherein when the amyloid binding compound of the present invention is structure C, then $R'^{2}$ is selected from the group consisting of a lower alkyl group, (CH$_3$)$_2$OR' (wherein n=1, 2, or 3), CF$_3$, CH$_2$-$CH_2$X, CH$_2$-$CH_2$-$CH_2$X (wherein X=F, Cl, Br or I), (C(O)=)$-$H, R$_{p/n}$, and (CH$_2$)$_n$R$_{p/n}$ wherein n=1, 2, 3, or 4; or

wherein when the amyloid binding compound of the present invention is structure D, then $R'^{2}$ is selected from the group consisting of (CH$_3$)$_2$OR' (wherein n=1, 2, or 3), CF$_3$, CH$_2$-$CH_2$X, CH$_2$-$CH_2$-$CH_2$X (wherein X=F, Cl, Br or I), (C(O)=)$-$H, R$_{p/n}$, and (CH$_2$)$_n$R$_{p/n}$ wherein n=1, 2, 3, or 4.

In another preferred embodiment, at least one of the substituents $R'^{2}$-$R'^{14}$ of the amyloid binding compound of the present invention is selected from the group consisting of $^{131}$I, $^{125}$I, $^{79}$Br, $^{83}$Br, $^{61}$F, CH$_3$-$CH_2$-X*, O-$CH_2$-$CH_2$-X*, CH$_3$-$CH_2$-$CH_2$-X*, O-$CH_2$-$CH_2$-$CH_2$-X*, (wherein X*=$^{131}$I, $^{125}$I, $^{79}$Br, $^{83}$Br or $^{61}$F), $^{18}$F, $^{15}$I, and a carbon-containing substituents as specified in the definition of the compounds having one of the structures A-E or F-J, wherein at least one carbon is $^{13}$C or $^{15}$C, a chelating group (with chelated metal group) of the form W-L* or Y-W-L*, wherein V is selected from the group consisting of $-\text{COO} -$,
$-\text{CO} -$,
$-\text{CH}_2\text{O} -$ and
—CH₂NH—; W is —(CH₂)n where n=0, 1, 2, 3, 4, or 5; and L* is:

\[
\text{or}
\]

wherein M* is \(^{99m}\text{Tc}\); and a chelating group (with chelated metal group) of the form W-L* or V-W-L*, wherein V is selected from the group consisting of —COO—, —CO—, —CH₂O— and —CH₂NH--; W is —(CH₂)n where n=0, 1, 2, 3, 4, or 5; and L* is:

\[
\text{or}
\]

wherein \(R^{15}\) independently is selected from one of the following:

\[
\text{or}
\]

and wherein \(R^{15}\) independently is selected from one of the following:

\[
\text{or}
\]
wherein Q is independently selected from one of the following structures:

![Chemical Structure Image]

wherein \( n = 0, 1, 2, 3 \) or 4,

![Chemical Structure Image 2]

wherein Z is \( S, N R', O \), or \( C(R')_2 \) in which \( R' \) is \( H \) or a lower alkyl group;

wherein U is \( N \) or \( CR' \);

wherein Y is \( NR^1 R^2, OR^2 \), or \( SR^2 \);

[0034] wherein each \( R^{17-24} \) independently is selected from the group consisting of \( H, F, Cl, Br, I, \) a lower alkyl group, \((CH_2)_n OR'\) (wherein \( n = 1, 2, \text{or } 3 \), \( CF_3 \), \( CH_2-CH_2X \), \( O-CH_2-CH_2X \), \( CH_2-CH_2-CH_2X \), \( O-CH_2-CH_2-CH_2X \), \( CH_2-CH_2-CH_2X \) (wherein \( X=F, Cl, Br \text{ or I} \), \( CN, (==O)-R', N(R')_2 \), \( NO_2 \), \( (==O)N(R')_2 \), \( O(CO)R', OR', SR', COOR', R^2R^3, CR^1-\text{CR}^1-\text{CR}^2-\text{CR}^2-\text{CR}^3-\text{CR}^3 \) (wherein \( R_{\text{ph}} \) represents an unsubstituted or substituted phenyl group with the phenyl substituents being chosen from any of the non-phenyl substituents denoted for \( R^{17-24} \) and wherein \( R' \) is \( H \) or a lower alkyl group).

[0035] In especially preferred embodiments, the compound is selected from structures A-E, and \( Z=S, Y=N, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \);

\( Z=S, Y=O, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \);

\( Z=S, Y=N, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \);

\( Z=S, Y=N, R^1=H, R^2=OH \) and \( R^2-R^7 \) and \( R^8-R^14 \) are \( H \);

\( Z=S, Y=O, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \);

\( Z=S, Y=N, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \);

\( Z=S, Y=O, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \);

or \( Z=S, Y=N, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \).

[0036] In especially preferred embodiments, the compound is selected from structures F-J, and \( Z=S, Y=N, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \);

\( Z=S, Y=O, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \);

\( Z=S, Y=N, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \);

or \( Z=S, Y=N, R^1=H, R^2=CH_3 \) and \( R^2-R^14 \) are \( H \).

[0037] In another preferred embodiment, at least one of the substituents \( R^3-R^14 \) is selected from the group consisting of \( O, N, OCH_2, OH \) and \( NH_2 \).

[0038] In still another preferred embodiment, the amyloid binding compound is selected from the group consisting of structure B, structure C and structure D; wherein \( R^1=H \), \( R^2=CH_3 \) and \( R^8 \) is selected from the group consisting of \( CN, CH_3, OH, OCH_2 \text{ and } NH_2 \); in a preferred aspect of this embodiment, \( R^2-R^7 \) and \( R^8-R^14 \) are \( H \).

[0039] In still another embodiment, the amyloid binding compounds of the present invention bind to \( \alpha \beta \) with a dissociation constant (Kd) between 0.0001 and 10.0 \( \mu \text{M} \) when measured by binding to synthetic \( \alpha \beta \) peptide or Alzheimer's Disease brain tissue.

[0040] Another embodiment of the invention relates to a method for synthesizing the amyloid binding compounds of the present invention having at least one of the substituents \( R^1-R^8 \) selected from the group consisting of \( 133, 135, 137, 76, 78, 75, 18, \text{and } 19 \); comprising the step of labeling the amyloid binding compound wherein at least one of the substituents \( R^1-R^8 \) is a tri-alkyl tin, by reaction of the compound with \( 133, 135, 137, 76, 78, 75, 18, \text{or } 19 \text{ containing substance.}

[0041] Another embodiment of the invention relates to a method for synthesizing the amyloid binding compounds of the present invention having at least one of the substituents \( R^1-R^8 \) selected from the group consisting of \( 133, 135, 137, 76, 78, 75, 18, \text{and } 19 \); comprising the step of labeling the amyloid binding compound of structure \( A-E \) or \( F-J \) wherein \( Z=S, Y=O, R^1=H \) and at least one of the substituents \( R^2-R^14 \) is a tri-alkyl tin, by reaction of the compound with \( 133, 135, 137, 76, 78, 75, 18, \text{or } 19 \text{ containing substance.}

[0042] A further embodiment of the present invention relates to a pharmaceutical composition for in vivo imaging of amyloid deposits, comprising (a) an amyloid binding compound chosen from the structures \( A-E \) or \( F-J \), and (b) a pharmaceutically acceptable carrier. A preferred aspect of the
embodiment relates to a pharmaceutical composition for in vivo imaging of amyloid deposits, comprising (a) an amyloid binding compound chosen from the structures A-E or F-J wherein Z=S, Y=N, R'=H, and (b) a pharmaceutically acceptable carrier.

[0043] In another embodiment of the invention is an in vivo method for detecting amyloid deposits in a subject, comprising the steps of: (a) administering a detectable quantity of a pharmaceutical composition comprising the labeled amyloid binding compound, and detecting the binding of the compound to amyloid deposit in the subject. In a preferred aspect of this embodiment, the amyloid deposit is located in the brain of a subject. In a particularly preferred aspect of this embodiment, the subject is suspected of having a disease or syndrome selected from the group consisting of Alzheimer's Disease, familial Alzheimer's Disease, Down's Syndrome and homozygotes for the apolipoprotein E4 allele. In another particularly preferred aspect of this embodiment, the detecting is selected from the group consisting of gamma imaging, magnetic resonance imaging and magnetic resonance spectroscopy. In a preferred aspect of this embodiment, the gamma imaging is either PET or SPECT. In another preferred aspect of this embodiment, the pharmaceutical composition is administered by intravenous injection. In another preferred aspect of this embodiment, the ratio of (i) binding of the compound to a brain area other than the cerebellum to (ii) binding of the compound to the cerebellum, in a subject, is compared to the ratio in a normal subject.

[0044] Another embodiment relates to a method of detecting amyloid deposits in biopsy or post-mortem human or animal tissue comprising the steps of: (a) incubating formalin-fixed or fresh-frozen tissue with a solution of an amyloid binding compound of the present invention to form a labeled deposit and then, (b) detecting the labeled deposits. In a preferred aspect of this embodiment, the solution is composed of 25-100% ethanol, with the remainder of the solution being water, wherein the solution is saturated with an amyloid binding compound according to the present invention. In a particularly preferred aspect of this embodiment, the solution is composed of an aqueous buffer (such as tris or phosphate) containing 0-50% ethanol, wherein the solution contains 0.0001 to 100 μM of an amyloid binding compound according to the present invention. In a particularly preferred aspect of this embodiment, the detecting is effected by microscopic techniques selected from the group consisting of bright-field, fluorescence, laser-confocal, and cross-polarization microscopy.

[0045] A further embodiment relates to a method of quantifying the amount of amyloid in biopsy or post-mortem tissue comprising the steps of: a) incubating a radiolabeled derivative of an amyloid binding compound of the present invention with a homogenate of biopsy or post-mortem tissue, wherein at least one of the substituents R'-R, of the compound is labeled with a radiolabel selected from the group consisting of 125I, 3H, and a carbon-containing substituent as specified by the amyloid binding compound structures A-E or F-J, wherein at least one carbon is 14C, b) separating the tissue-bound from the tissue-unbound radiolabeled derivative of an amyloid binding compound of the present invention, c) quantifying the tissue-bound radiolabeled derivative of an amyloid binding compound of the present invention, and d) converting the units of tissue-bound radiolabeled derivative of an amyloid binding compound of the present invention to units of micrograms of amyloid per 100 mg of tissue by comparison with a standard.

[0046] In a preferred aspect of the above embodiment, the radiolabeled derivative of the amyloid binding compound of the present invention or a water soluble, non-toxic salt thereof is according to one of the formulae A-E below:

\[
\text{Structure A} \\
\text{Structure B} \\
\text{Structure C} \\
\text{Structure D} \\
\text{Structure E}
\]

wherein Z is S, NR', O or C(R')₂, in which case the correct tautomeric form of the heterocyclic ring becomes an indole in which R' is H or a lower alkyl group.
wherein \( Y \) is \( NR'R^2 \), \( OR^2 \), or \( SR^2 \);

wherein the nitrogen of any group is not a quaternary amine;

or the radiolabeled derivative of the amyloid binding compound of the present invention or a water soluble, non-toxic salt thereof is according to one of the formulae F-J below:

- **Structure F**

- **Structure G**

- **Structure H**

wherein each \( Q \) is independently selected from one of the following structures:
wherein $Y$ is NR'R, OR', or SR'; wherein the nitrogen of

![Chemical structure](image)

is not a quaternary amine; wherein each $R'$ and $R''$ independently is selected from the group consisting of $H$, a lower alkyl group, $(CH_2)_nOR'$ (wherein $n=1$, 2, or 3), $CF_3$, $CH_2—CH_2X$, $CH_2—CH_2—CH_2X$ (wherein $X$=F, Cl, Br or I), $(C=O)—R'$, $R_{pds}$- and $(CH_2)_nR_{pds}$ (wherein $n=1, 2, 3, 4$ and $R_{pds}$ represents an unsubstituted or substituted phenyl group with the phenyl substituents being chosen from any of the non-phenyl substituents defined below for $R^3-R'^3$ and $R'$ is $H$ or a lower alkyl group); and wherein each $R^3-R'^3$ independently is selected from the group consisting of $H$, $F$, $Cl$, $Br$, or $I$, a lower alkyl group, $(CH_2)_nOR'$ (wherein $n=1$, 2, or 3), $CF_3$, $CH_2—CH_2X$, $CH_2—CH_2—CH_2X$, $O—CH_2—CH_2X$, $O—CH_2—CH_2—CH_2X$ (wherein $X$=F, Cl, Br or I), $CN$, $(C=O)—R'$, $N(R')_2$, $NO_2$, $(C=O)N(R')_2$, $O(CO)R'$, $OR'$, $SR'$, $COOR'$, $R_{pds}$- $CR—CR—CR—R_{pds}$, $CR—CR—CR—CR—CR—R_{pds}$ (wherein $R_{pds}$ represents an unsubstituted or substituted phenyl group with the phenyl substituents being chosen from any of the non-phenyl substituents defined for $R^3-R'^3$ and wherein $R'$ is $H$ or a lower alkyl group), a tri-alkyl tin and a chelating group (with or without a chelated metal group) of the form $W—L$ or $V—W—L$, wherein $V$ is selected from the group consisting of $—CO—$, $—CO—CH_2O—$ and $—CH_2NH—$; $W$ is $—(CH_2)_n$ where $n=0, 1, 2, 3, 4$, or 5; and $L$ is:

![Chemical structure](image)

wherein $M$ is selected from the group consisting of Tc and Re; or wherein each $R^1$ and $R^2$ is a chelating group (with or without a chelated metal group) of the form $W—L$, wherein $V$ is selected from the group consisting of $—CO—$, and $—CO—$; $W$ is $—(CH_2)_n$ where $n=0, 1, 2, 3, 4$, or 5; and $L$ is:

![Chemical structure](image)
and wherein $R'$ independently is selected from the following:

$$
\begin{align*}
H, & \quad \text{COOH,} \\
\text{CONHCH}_3, & \quad \text{CH}_3,
\end{align*}
$$

or an amyloid binding, chelating compound (with or without a chelated metal group) or a water soluble, non-toxic salt thereof of the form:

$$
\begin{align*}
\text{or } & \quad \text{or } \\
\text{or } & \quad \text{or }
\end{align*}
$$

wherein $R'^{15}$ independently is selected from the following:

$$
\begin{align*}
H, & \quad \text{COOH,} \\
\text{CONHCH}_3, & \quad \text{CH}_3,
\end{align*}
$$

and $R'^{16}$ is

[0048]
wherein Z is S, NR', O, or (C(R'))₂ in which R' is H or a lower alkyl group;
wherein U is N or CR';
wherein Y is NR'R, OR, or (C(R'))₂;
wherein each R₁⁻⁴⁻Rₙ₄⁻ independently is selected from the group consisting of H, F, Cl, Br, I, a lower alkyl group, (CH₂)nOR' (wherein n=1, 2, or 3), CF₃, CH₂=CHX, O—CH₂—CH₃, CH₃—CH₂—CHX, O—CH₂—CH₃—
CH₂X (wherein X=F, Cl, Br or I), CN, (C==O)—R₁⁻⁴⁻N(R')₂, NO₂, (C==O)N(R')₂, O(COR')₂, OR', SR', COOR', R₂⁻⁴⁻CR⁻¹⁻R₉⁻₂⁻ and CR₂⁻¹⁻R₉⁻₁⁻ (wherein R₉⁻₁⁻ represents an unsubstituted or substituted phenyl group with the phenyl substituents being chosen from any of the non-phenyl substituents defined for R₁⁻⁴⁻Rₙ₄⁻ and wherein R' is H or a lower alkyl group).

[0049] Another embodiment relates to a method of distinguishing an Alzheimer’s disease brain from a normal brain comprising the steps of: a) obtaining tissue from (i) the cerebellum and (ii) another area of the same brain other than the cerebellum, from normal subjects and from subjects suspected of having Alzheimer’s disease; b) incubating the tissues with a radiolabeled derivative of a thioflavin amyloid binding compound according to the present invention so that amyloid in the tissue binds with the radiolabeled derivative of an amyloid binding compound of the present invention; c) quantifying the amount of amyloid bound to the radiolabeled derivative of an amyloid binding compound of the present invention according to the above recited method; d) calculating the ratio of the amount of amyloid in the area of the brain other than the cerebellum to the amount of amyloid in the cerebellum; e) comparing the ratio for amount of amyloid in the tissue from normal subjects with ratio for amount of amyloid in tissue from subjects suspected of having Alzheimer’s disease; and i) determining the presence of Alzheimer’s disease if the ratio from the brain of a subject suspected of having Alzheimer’s disease is above 90% of the ratios obtained from the brains of normal subjects.

[0050] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims. Additionally, all documents referred to herein are expressly incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0051] FIG. 1 Shows the structures of a Thioflavin S and Thioflavin T;
[0052] FIG. 2 Shows the structures of two thioflavin derivatives according to the invention;
[0053] FIG. 3 Shows four serial sections of fluorescent dyed brain frontal cortex of an AD patient;
[0054] FIG. 4 Shows proposed sites of binding of Chrysamine G and Thioflavin T α-synuclein fibrils;
[0055] FIG. 5 Shows competition assay using Chrysamine G, Thioflavin S and Thioflavin T, and derivatives of the present invention (BTA-0, BTA-1 and BTA-2);
[0056] FIG. 6 Shows time course radioactivity in the frontal cortex of baboons injected with labeled BTA-1,6-Me-BTA-1 and 6-Me-BTA-1; and
[0057] FIG. 7 Shows a transverse positron emission tomography image of two levels of baboon brain following i.v. injection of N-methyl-1C]BTA-1.

[0058] FIG. 8 Shows post-mortem sections of human and transgenic mouse brain stained with a derivative of the present invention (BTA-1).

[0059] FIG. 9 Shows in vivo labeling of amyloid plaques and vascular amyloid stained by a derivative of the present invention (BTA-1) in living transgenic mice imaged with multiphoton microscopy.

DETAILED DESCRIPTION OF THE INVENTION

[0060] The present invention exploits the ability of Thioflavin compounds and radiolabeled derivatives thereof to cross the blood brain barrier in vivo and bind to Aβ in neurotic (but not diffuse) plaques, to Aβ deposited in cerebrovascular amyloid, and to the amyloid consisting of the protein deposited in NFT. The present compounds are non-quaternary amine derivatives of Thioflavin S and T which are known to stain amyloid in tissue sections and bind to synthetic Aβ in vitro. Kelenyi J. Histochim. Cytochem.: 15; 172 (1967); Burns et al. J. Path. Bact.: 94:337 (1967); Guntern et al. Experientia: 48:8 (1992); I.eVine Meth. Enzymol. 309:274 (1999).

[0061] The thioflavin derivatives of the present invention have each of the following characteristics: (1) specific binding to synthetic Aβ in vitro and (2) ability to cross a non-compromised blood brain barrier in vivo.

[0062] As used herein to describe the thioflavin derivatives, “lower alkyl” is branched or straight chain C₃-C₆, preferably C₄-C₆, and most preferably C₅-C₆ (e.g., methyl, ethyl, propyl or butyl). When R⁻¹⁻Rₙ₄⁻ is defined as “tri-alkyl” tine, the moiety is a tri-C₃-C₆ alkyl Sn moiety; preferably tri-C₃-C₄ alkyl Sn moiety, most preferably tri-C₃-C₄ alkyl Sn moiety (e.g., methyl, ethyl, propyl or butyl).

[0063] The method of this invention determines the presence and location of amyloid deposits in an organ or body area, preferably brain, of a patient. The present method comprises administration of a detectable quantity of a pharmaceutical composition containing an amyloid binding compound chosen from structures A-E or F-J, as defined above, called a “detectable compound,” or a pharmaceutically acceptable water-soluble salt thereof, to a patient. A “detectable quantity” means that the amount of the detectable compound that is administered is sufficient to enable detection of binding of the compound to amyloid. An “imaging effective quantity” means that the amount of the detectable compound that is administered is sufficient to enable imaging of binding of the compound to amyloid.

[0064] The invention employs amyloid probes which, in conjunction with non-invasive neuroimaging techniques such as magnetic resonance spectroscopy (MRS) or imaging (MRI), or gamma imaging such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT), are used to quantify amyloid deposition in vivo. The term “in vivo imaging” refers to any method which permits the detection of a labeled thioflavin derivative which is chosen from structures A-E or F-J, as described above. For gamma imaging, the radiation emitted from the organ or area being examined is measured and expressed either as total binding or as a ratio in which total binding in one tissue is normalized to (for example, divided by) the total binding in another tissue of the same subject during the same in vivo imaging procedure. Total binding in vivo is defined as the entire signal detected in a tissue by an in vivo imaging technique without the need for correction by a second injection of an identical quantity of labeled compound along with a large
excess of unlabeled, but otherwise chemically identical compound. A “subject” is a mammal, preferably a human, and most preferably a human suspected of having dementia.

[0065] For purposes of in vivo imaging, the type of detection instrument available is a major factor in selecting a given label. For instance, radioactive isotopes and 19F are particularly suitable for in vivo imaging in the methods of the present invention. The type of instrument used will guide the selection of the radionuclide or stable isotope. For instance, the radioactive isotope chosen must have a type of decay detectable by a given type of instrument. Another consideration relates to the half-life of the radionuclide. The half-life should be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that the host does not sustain dangerous radiation. The radionuclide chosen for the invention can be detected using gamma imaging wherein emitted gamma radiation of the appropriate wavelength is detected. Methods of gamma imaging include, but are not limited to, SPECT and PET. Preferably, for SPECT detection, the chosen radionuclide will lack a particulate emission, but will produce a large number of photons in a 140-200 keV range. For PET detection, the radionuclide will be a positron-emitting radionuclide such as 19F which will annihilate to form two 511 keV gamma rays which will be detected by the PET camera.

[0066] In the present invention, amyloid binding compounds/probes are made which are useful for in vivo imaging and quantification of amyloid deposition. These compounds are to be used in conjunction with non-invasive neuroimaging techniques such as magnetic resonance spectroscopy (MRS) or imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT). In accordance with this invention, the thioflavin derivatives may be labeled with 19F, 75Br, or 19F for PET by techniques well-known in the art and are described by Fowler, J. and Wolf, A. in POSITRON EMISSION TOMOGRAPHY AND AUTORADIOGRAPHY (Phelps, M., Mazziotta, J., and Selbert, H. eds.) 391-450 (Raven Press, NY 1986) the contents of which are hereby incorporated by reference. The thioflavin derivatives also may be radiolabeled with 19F, 75Br, or 19F for PET by techniques well-known in the art and are described by Fowler, J. and Wolf, A. in POSITRON EMISSION TOMOGRAPHY AND AUTORADIOGRAPHY (Phelps, M., Mazziotta, J., and Selbert, H. eds.) 391-450 (Raven Press, NY 1986) the contents of which are hereby incorporated by reference. The thioflavin derivatives also may be radiolabeled with 19F for SPECT by any of several techniques known to the art. See, e.g., Kulkarni, Int. J. Rad. Appl. & Instr. (Part B) 18: 647 (1991), the contents of which are hereby incorporated by reference. In addition, the thioflavin derivatives may be labeled with any suitable radioactive iodine isotope, such as, but not limited to, 131I, 125I, or 123I, by iodination of a diazotized amino derivative directly via a diazonium iodide, see Greenbaum, F. Am. J. Pharm. 108: 17 (1936), or by conversion of the unstable diazotized amine to the stable triazine, or by conversion of a non-radioactive halogenated precursor to a stable tri-alkyl tin derivative which then can be converted to the iodo compound by several methods well-known to the art. See, Satyamurthy and Barrio J. Org. Chem. 48: 4394 (1983), Goodman et al., J. Org. Chem. 49: 2322 (1984), and Mathis et al., J. Labelled Comp. and Radiopharm. 1994: 905; Chumpradit et al., J. Med. Chem. 34: 877 (1991); Zhuang et al., J. Med. Chem. 37: 1406 (1994); Chumpradit et al., J. Med. Chem. 37: 4245 (1994). For example, a stable triazine or tri-alkyl tin derivative of thioflavin or its analogues is reacted with a halogenating agent containing 131I, 125I, 123I, 75Br, 79Br, 18F, or 19F. Thus, the stable tri-alkyl tin derivatives of thioflavin and its analogues are novel precursors useful for the synthesis of many of the radiolabeled compounds within the present invention. As such, these tri-alkyl tin derivatives are one embodiment of this invention.

[0067] The thioflavin derivatives also may be radiolabeled with known metal radiolabels, such as Technetium-99m (99mTc). Modification of the substituents to introduce ligands that bind such metal ions can be effected without undue experimentation by one of ordinary skill in the radiolabeling art. The metal radiolabeled thioflavin derivative can then be used to detect amyloid deposits. Preparing radiolabeled derivatives of 99mTc is well known in the art. See, for example, Zhuang et al., “Neutral and stereospecific Tc-99m complexes: [99 mTc]N-benzyl-3,4-di-(N-2-mercaptoprolyl)-amino-pyroridines (P-BAT)” Nuclear Medicine & Biology 26(2):217-24, (1999); Oya et al., “Small and neutral Tc(v)O BAT, bisaminoethanol (N2S2) complexes for developing new brain imaging agents” Nuclear Medicine & Biology 25(2):135-40, (1998); and Hom et al., “Technetium-99m-labeled receptor-specific small-molecule radiopharmaceuticals: recent developments and encouraging results” Nuclear Medicine & Biology 24(6):485-98, (1997).

[0068] The methods of the present invention may use isotopes detectable by nuclear magnetic resonance spectroscopy for purposes of in vivo imaging and spectroscopy. Elements particularly useful in magnetic resonance spectroscopy include 19F and 13C.

[0069] Suitable radioisotopes for purposes of this invention include beta-emitters, gamma-emitters, positron-emitters, and x-ray emitters. These radioisotopes include 131I, 125I, 18F, 11C, 75Br, 79Br, Suitable stable isotopes for use in Magnetic Resonance Imaging (MRI) or Spectroscopy (MRS), according to this invention, include 19F and 13C. Suitable radioisotopes in vitro quantification of amyloid in homogenates of biopsy or post-mortem tissue include 125I, 13C, and 1H. The preferred radiolabels are 13C or 19F for use in PET in vivo imaging, for use in SPECT imaging, 19F for MRS/MRI, and 1H or 13C for in vitro studies. However, any conventional method for visualizing diagnostic probes can be utilized in accordance with this invention.

[0070] The method may be used to diagnose AD in mild or clinically confusing cases. This technique would also allow longitudinal studies of amyloid deposition in human populations at high risk for amyloid deposition such as Down’s syndrome, familial AD, and homozygous for the apolipoprotein E4 allele. Corder et al., Science 261: 921 (1993). A method that allows the temporal sequence of amyloid deposition to be followed can determine if deposition occurs long before dementia begins or if deposition is unrelated to dementia. This method can be used to monitor the effectiveness of therapies targeted at preventing amyloid deposition.

[0071] Generally, the dosage of the detectably labeled thioflavin derivative will vary depending on considerations such as age, condition, sex, and extent of disease in the patient, contraindications, if any, concomitant therapies and other variables, to be adjusted by a physician skilled in the art. Dosage can vary from 0.001 µg/kg to 10 µg/kg, preferably 0.01 µg/kg to 1.0 µg/kg.

[0072] Administration to the subject may be local or systemic and accomplished intravenously, intraarterially, intrathecellically (via the spinal fluid) or the like. Administration
may also be intradermal or intracavitary, depending upon the body site under examination. After a sufficient time has elapsed for the compound to bind with the amyloid, for example 30 minutes to 48 hours, the area of the subject under investigation is examined by routine imaging techniques such as MRS/MRI, SPECT, planar scintillation imaging, PET, and any emerging imaging techniques, as well. The exact protocol will necessarily vary depending upon factors specific to the patient, as noted above, and depending upon the body site under examination, method of administration and type of label used; the determination of specific procedures would be routine to the skilled artisan. For brain imaging, preferably, the amount (total or specific binding) of the bound radioactively labeled thioflavin derivative or analogue of the present invention is measured and compared (as a ratio) with the amount of labeled thioflavin derivative bound to the cerebellum of the patient. This ratio is then compared to the same ratio in age-matched normal brain.

[0073] The pharmaceutical compositions of the present invention are advantageously administered in the form of injectable compositions, but may also be formulated into well known drug delivery systems (e.g., oral, rectal, parenteral (intravenous, intramuscular, or subcutaneous), intraesternal, intravaginal, intraperitoneal, local (powders, ointments or drops), or as a buccal or nasal spray). A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the composition may contain about 10 to 50 mg of human serum albumin and from about 0.5 to 500 micrograms of the labeled thioflavin derivative per milliliter of phosphate buffer containing NaCl. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in REMINGTON'S PHARMACEUTICAL SCIENCES, 15th Ed. Easton: Mack Publishing Co. pp. 1405-1412 and 1461-1487 (1975) and THE NATIONAL FORMULARY XIV., 14th Ed. Washington: American Pharmaceutical Association (1975), and the UNITED STATES PHARMACOPEIA XVIII. 18th Ed. Washington: American Pharmaceutical Association (1995), the contents of which are hereby incorporated by reference.

[0074] Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobials, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See, Goodman and Gilman's THE PHARMACOLOGICAL BASIS FOR THERAPEUTICS (7th Ed.).

[0075] Particularly preferred pharmaceutical compositions of the present invention are those that, in addition to specifically binding amyloid in vivo and capable of crossing the blood brain barrier, are also non-toxic at appropriate dosage levels and have a satisfactory duration of effect.

[0076] According to the present invention, a pharmaceutical composition comprising thioflavin amyloid binding compounds, is administered to subjects in whom amyloid or amyloid fibril formation are anticipated. In the preferred embodiment, such subject is a human and includes, for instance, those who are at risk of developing cerebral amyloid, including the elderly, nondemented population and patients having amyloidosis associated diseases and Type 2 diabetes mellitus. The term “preventing” is intended to include the amelioration of cell degeneration and toxicity associated with fibril formation. By “amelioration” is meant the treatment or prevention of more severe forms of cell degeneration and toxicity in patients already manifesting signs of toxicity, such as dementia.

[0077] The pharmaceutical composition comprises thioflavin amyloid binding compounds described above and a pharmaceutically acceptable carrier. In one embodiment, such pharmaceutical composition comprises serum albumin, thioflavin amyloid binding compounds and a phosphate buffer containing NaCl. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in REMINGTON'S PHARMACEUTICAL SCIENCES, 15th Ed. Easton: Mack Publishing Co., pp. 1405-1412 and 1461-1487 (1975) and THE NATIONAL FORMULARY XIV., 14th Ed. Washington: American Pharmaceutical Association (1975), and the UNITED STATES PHARMACOPEIA XVIII. 18th Ed. Washington: American Pharmaceutical Association (1995), the contents of which are hereby incorporated by reference.

[0078] Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobials, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See, Goodman and Gilman's THE PHARMACOLOGICAL BASIS FOR THERAPEUTICS (7th Ed.).

[0079] The inventive pharmaceutical composition could be administered orally, in the form of a liquid or solid, or injected intravenously or intramuscularly, in the form of a suspension or solution. By the term “pharmaceutically effective amount” is meant an amount that prevents cell degeneration and toxicity associated with fibril formation. Such amount would necessarily vary depending upon the age, weight and condition of the patient and would be adjusted by those of ordinary skill in the art according to well-known protocols. In one embodiment, a dosage would be between 0.1 and 100 mg/kg per day, or divided into smaller dosages to be administered two to four times per day. Such a regimen would be continued on a daily basis for the life of the patient. Alternatively, the pharmaceutical composition could be administered intramuscularly in doses of 0.1 to 100 mg/kg every one to six weeks.

[0080] According to the aspect of the invention which relates to a method of detecting amyloid deposits in biopsy or post-mortem tissue, the method involves incubating formalin-fixed tissue with a solution of a thioflavin amyloid binding compound chosen from structures A-E or F-J, described above. Preferably, the solution is 25-100% ethanol, with the remainder being water, saturated with a thioflavin amyloid binding compound according to the invention. Upon incubation, the compound stains or labels the amyloid deposit in the tissue, and the stained or labeled deposit can be detected or visualized by any standard method. Such detection means include microscopic techniques such as bright-field, fluorescence, laser-confocal and cross-polarization microscopy.

[0081] The method of quantifying the amount of amyloid in biopsy or post-mortem tissue involves incubating a labeled
derivative of thioflavin according to the present invention, or a water-soluble, non-toxic salt thereof, with homogenate of biopsy or post-mortem tissue. The tissue is obtained and homogenized by methods well known in the art. The preferred label is a radiolabel, although other labels such as enzymes, chemiluminescent and immunofluorescent compounds are well known to skilled artisans. The preferred radiolabel is $^{125}$I, $^{14}$C or $^3$H, the preferred label substituent of an amyloid binding compound chosen from structures A-E or F-J is at least one of $^2$R-$^3$R. Tissue containing amyloid deposits will bind to the labeled derivatives of the thioflavin amyloid binding compounds of the present invention. The bound tissue is then separated from the unbound tissue by any mechanism known to the skilled artisan, such as filtering. The bound tissue can then be quantified through any means known to the skilled artisan. The units of tissue-bound radiolabeled thioflavin derivative are then converted to units of micrograms of amyloid per 100 mg of tissue by comparison to a standard curve generated by incubating known amounts of amyloid with the radiolabeled thioflavin derivative.

The method of distinguishing an Alzheimer’s diseased brain from a normal brain involves obtaining tissue from (i) the cerebellum and (ii) another area of the same brain, other than the cerebellum, from normal subjects and from subjects suspected of having Alzheimer’s disease. Such tissues are made into separate homogenates using methods well known to the skilled artisan, and then are incubated with a radiolabeled thioflavin amyloid binding compound. The amount of tissue which binds to the radiolabeled thioflavin amyloid binding compound is then calculated for each tissue type (e.g. cerebellum, non-cerebellum, normal, abnormal) and the ratio for the binding of non-cerebellum to cerebellum tissue is calculated for tissue from normal tissue and for tissue from patients suspected of having Alzheimer’s disease. These ratios are then compared. If the ratio from the brain suspected of having Alzheimer’s disease is above 90% of the ratios obtained from normal brains, the diagnosis of Alzheimer’s disease is made. The normal ratios can be obtained from previously obtained data, or alternatively, can be recalculate at the same time the suspected brain tissue is studied.

**Molecular Modeling**

Molecular modeling was done using the computer modeling program Alchemy2000 Tripost, Inc. St. Louis, Mo.) to generate the Aβ peptide chains in the anti-parallel beta-sheet conformation. Kirschner et al., Proc. Natl. Acad. Sci. U.S.A. 83: 503 (1986). The amyloid peptides were placed in hairpin loops (Hilbich et al., J. Mol. Biol. 218: 149 (1991)) and used without further structural refinement. The Aβ peptides were aligned so that alternate chains were spaced 4.76 A apart, characteristic of beta-sheet fibrils. Kirschner, supra.

Preparation of Thioflavin Derivatives for Tissue Staining

Both Thioflavin S (ThS) and Thioflavin T (ThT) were utilized as pharmacophores (see, e.g., FIG. 1). It is noted that both compounds contain quaternary amines and are, therefore, quite hydrophilic as a result.

[C-14]ThT was synthesized and used to determine relative lipophilicity by partitioning between octanol and phosphate-buffered saline. The log of the partition coefficient, log $P_{oct}$, was found to be 0.57 for [C-14]ThT. It was determined that the quaternary amine renders ThT too polar for use as an effective brain imaging agent. Based on the results of lipophilic Congo red derivatives (phenols uncharged at physiologic pH, but potentially ionizable with a $pK_a$ of ~8.5) (Klunk et al. WO09634853A1, WO09847969A1, WO09924394A2), the inventors removed the methyl group from the benzothiazole nitrogen for the ThT derivatives. The removal of the methyl moiety eliminated the charged quaternary amine from the heterocyclic portion of the molecule, leaving an aromatic amine which typically have $pK_a$ values ~5.5. Shorthand nomenclature for the ThT derivative is used wherein the basic backbone is designated BTA (for Benzo[1]thiazole-Aniline). Substituents on the benzothiazole ring are placed before the ‘B’ and the number of methyl groups on the aniline nitrogen is placed after the ‘A’ (see, e.g., FIG. 2).

**Molecular Staining with ThT and Derivatives**

ThT (see, e.g., FIG. 1) is a fluorescent dye that has been used as a histological stain for amyloid (Burns et al., “The specificity of the staining of amyloid deposits with thioflavine T” Journal of Pathology & Bacteriology 94:337-344; 1967.). ThT weakly stains plaques (see, e.g., FIG. 3), tangles, neuritip threads and cerebrovascular amyloid (CVA) in AD brain. Preliminary tissue staining shows that both the primary amine 2-(4-aminophenyl)-6-methyl-benzothiazole (6-Me-BTA-6) and the tertiary amine 2-(4-dimethylamino phenyl)-6-methyl-benzothiazole (6-Me-BTA-2) also stain plaques and tangles in post-mortem AD brain (see, e.g., FIG.

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</table>
3). Experiments in which the concentrations of 6-Me-BTA-0 and 6-Me-BTA-2 were progressively decreased showed that staining by both 6-Me-BTA-0 and 6-Me-BTA-1 could still be detected with staining solutions containing only 10 nM of the BTA compound. In contrast, BTP (2-phenylbenzo(thiazole) does not appear to stain plaques, however, this compound is not nearly as fluorescent as the BTA derivatives. Thus, in the development of these compounds, tissue staining has served the dual purpose of assessing specificity of staining in AD brain tissue as well as assessing binding affinity by screening staining solutions over a range of concentrations similar to that employed in the binding assays.

[0089] ii. Binding Models of Congo Red Derivatives and ThT to Aβ

[0090] There are some theories about the binding mechanism of ThT to β-amyloid, but no specific theory has been proven or accepted. However, the mechanism appears to be specific and saturable (LeVine, “Quantification of beta-sheet amyloid fibril structures with thioflavin T” Method. Enzymol. 309:272-284; 1999). Thus, it is possible to localize the potential binding site(s) on Aβ and develop a binding model in a manner analogous to that used to develop the Congo red (CR)/Chrysamine-G (CG) binding model (Klunk et al., “Developments of small molecule probes for the beta-amyloid protein of Alzheimer’s disease” Neurobiol. Aging 15:691-698; 1994.) based on the following structural and binding properties. First, ThT and CG have opposite charges at physiological pH, and it is unlikely that they share a common binding site. This is supported by the lack of competition of ThT for [1H]CG binding to Aβ fibrils (see, e.g., FIG. 5).

[0091] Previous structural studies of Aβ fibrils (Hilbich et al., “Aggregation and secondary structure of synthetic amyloid beta A4 peptides of Alzheimer’s disease” Journal of Molecular Biology 218:149-63; 1991.) and CR and CG binding to Aβ fibrils suggested a molecular model in which CG binds through a combination of electrostatic and hydrophobic interaction to the area of Lys-16 (see, e.g., FIG. 4). The studies of LeVine (LeVine ibid) help localize the site of ThT binding to Aβ by showing that ThT binds well to Aβ12-28, but negligibly to Aβ25-35. This suggests the ThT binding site lies somewhere between residues 12 and 24 of Aβ. It is likely that the positively charged ThT (a quaternary amine) will be attracted to negatively charged (acidic) residues on Aβ. However, the acidic residues are Glu-22 and Asp-23. While both of these are candidates, the existing model predicts that Glu-22 is involved very near the Lys-16 binding site for CG. The current “working” model localizes ThT binding to the area of Asp-23—on the opposite side of the fibril from the proposed CG site. Since the key feature of ThT (and CG) binding is the presence of a beta-sheet fibril, binding must require more than just a single amino acid residue. The binding site exists when residues not normally interacting in monomers are brought together in the beta-sheet fibril. Therefore, without being bound to any one theory, it is believed that ThT also interacts via hydrogen bonds to His-13 and Gln-15 of a separate, adjacent Aβ molecule comprising the beta-sheet fibril.

[0092] iii. Radiolabeling of ThT and Radioligand Binding Assays

[0093] Assessing binding by tissue staining is useful, particularly for assessing specificity.

[0094] The compound BTP, which is not very fluorescent, may not show staining either because it does not bind well enough, or because it is not fluorescent enough. In addition to

the AD tissue staining, quantitative binding assays can be conducted spectrophotometrically (LeVine ibid). This assay depends on metachromatic spectral shift which occurs when ThT binds to the amyloid fibril. While this assay can be useful to individually screen highly fluorescent compounds that show this metachromatic shift, it has not been determined to be useful for competition assays. For example, it is commonly observed that test compounds (e.g., CG) quench the fluorescence of the ThT-Aβ complex (as well as ThT alone). Compounds that quench, but do not bind to the ThT site, will falsely appear to bind. Therefore, it is preferable to use radio-labeled ThT in typical radioligand binding assays with aggregated Aβ. In this assay, inhibition of radiolabeled ThT binding to Aβ trapped on filters would represent true inhibition of ThT binding and does not require the test compound to be highly fluorescent.

[0095] The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available document, including U.S. patents, are specifically incorporated into this patent application by reference.

EXAMPLES

[0096] All of the reagents used in the synthesis were purchased from Aldrich Chemical Company and used without further purification. Melting points were determined on Mel-Temp II and were uncorrected. The 1H NMR spectra of all compounds were measured on Bruker 300 using TMS as internal reference and were in agreement with the assigned structures. The TLC was performed using Silica Gel 60 F254 from EM Sciences and detected under UV lamp. Flash chromatography was performed on silica gel 60 (230-400 mesh, purchased from Mallinckrodt Company. The reverse phase TLC were purchased from Whiteman Company.

Synthesis Examples

Example 1

Synthesis of Primuline Base Derivatives

[0097] Route 1: Example of the synthesis of Primuline compounds is according to the reaction scheme shown below:
The primuline derivatives are prepared based on Schubert's method (Schubert, M. Zur Kenntnis der Dehydrotiolauldin- and Primulin-sulfosäuren, Justus Liebigs Ann. Chem. 558, 10-33, 1947) through condensation of 2-amino-5-methylthiophenol with 2-(p-nitrophenyl)-benzothiazole-6-carboxylic chloride and subsequent reduction of the nitro group with tin chloride in ethanol. Substituted derivatives of primuline base are synthesized with the appropriate substituted p-nitrobenzoylchlorides and R²-Rº substituted 2-aminothiophenol.

Following the same strategy as above, the other claimed primulin derivatives may be synthesized by substituting the appropriate substituted 3-mercapto-4-amino benzoic acid derivative (e.g. 2-, 5-, or 6-methyl-3-mercapto-4-amino benzoic acid), the appropriate 4-nitro-benzyol chloride derivative (e.g. 2- or 3-methyl-4-nitro-benzyol chloride) or the appropriate 2-amino-5-methylthiophenol derivative (e.g. 3,5-, 4,5-, or 5,6-dimethyl-2-aminothiophenol).

**Example 2**

Synthesis of 2-[2-(4'-amino phenyl)ethylenyl]-benzothiazole derivatives

**Route 3:** Example of the synthesis of BTEA-0.1.2 and BTAA-0.1.2, which are representative of the group of BTEA and BTAA compounds was according to the reaction scheme shown below:

(a) Trans-2-(4'-Nitrophenylethenyl)benzothiazole

trans-4-Nitrocinnamyl chloride 10 (1.77 g, 9.5 mmol, 1.2 eq.) in DMF (20 ml) was added dropwise to a solution of 2-aminothiophenol 9 (1.0 g, 8.0 mmol) in DMF
(15 ml) at room temperature. The reaction mixture was stirred at room temperature for overnight. The reaction mixture was poured into a solution of 10% sodium carbonate (100 ml). The participate was collected by filtration under reduced pressure. Recrystallization from methanol gave 1.92 g (85.1%) of the product 11.

(b) 2-(4-Aminophenylethenyl)benzothiazole (12)

[0102] A mixture of 2-(4-nitrophenoxy)benzothiazole (11) (500 mg, 1.7 mmol) and tin(II) chloride dihydrate (1.18 g, 5.2 mmol) in anhydrous ethanol (20 ml) was refluxed under N₂ for 4 hrs. After concentration of the filtrate gave 7 mg (58%) of the product 12.

(c) 2-(4-Methylaminophenylethenyl)benzothiazole (13)

[0103] A mixture of 2-(4-aminophenylethenyl)benzothiazole (12) (7 mg), Mel (3.9 mg) and anhydrous K₂CO₃ (100 mg) in DMSO (anhydrous, 0.5 ml) was heated at 100°C for 16 hrs. The reaction mixture was purified by reverse phase TLC (MeOH:CH₂Cl₂=7:1) to give 2.5 mg (32.7%) of the product 13.

(d) 2-(4-Aminophenylethenyl)benzothiazole (14)

[0104] 2-(4-Nitrophenoxy)benzothiazole (30 mg, 0.10 mmol) was dissolved in MeOH (10 ml). Pd/C (10%, 40 mg) was added and the reaction mixture was stirred under H₂ atmosphere at room temperature 60 hrs. The catalyst was filtrated and washed with methanol (ca. 2 ml). Evaporation of the filtrate gave the crude product which was purified with TLC (hexanes:ethyl acetate=70:40, 1:1) to give 15 mg (50%) of the product. ¹H NMR (300 MHz, MeOD-d₄) δ: 7.88 (d, J=8.3 Hz, 1H, H-7), 7.86 (d, J=8.1 Hz, 1H, H-4), 7.48 (dd, J₁,J₂=6.2 Hz, 1H, H-5 or H-6), 7.38 (dd, J₁,J₂=8.2 Hz, 1H, H-5 or H-6), 6.96 (d, J=6.8 Hz, 2H, H-2',6'), 6.62 (d, J=6.8 Hz, 2H, H-3', 5'), 3.36 (t, J=7.4 Hz, 2H, CH₂), 3.03 (t, J=7.4 Hz, 2H, CH₂).

(e) 2-(4-Dimethylaminophenylethenyl)benzothiazole (16)

[0105] A mixture of 2-aminophenol (0.51 g, 4.1 mmol), trans-4-dimethylaminocinnamic acid (14) (0.79 g, 4.1 mmol) and PPA (10 g) was heated to 220°C for 4 hrs. The reaction mixture was cooled to room temperature and poured into 10% of potassium carbonate solution (~400 ml). The residue was collected by filtration under reduced pressure. Purification with flash column (hexanes:ethyl acetate=2:1) gave 560 mg (48.7%) of product 15 as a yellow solid.

(f) 2-(4-Dimethylaminophenylethenyl)benzothiazole (17)

[0106] 2-(4-Dimethylaminophenylethenyl)benzothiazole (12 mg, 0.038 mmol) was dissolved in MeOH (5 ml). Pd/C (10%, 20 mg) was added and the reaction mixture was stirred under H₂ atmosphere at room temperature 16 hr. The catalyst was filtrated and washed with methanol (ca. 1 ml). Evaporation of the filtrate gave 7 mg (58%) of the product. ¹H NMR (300 MHz, Acetone-d₆) δ: 7.97 (d, J=8.3 Hz, 1H, H-7), 7.93
(a) 4-Methoxy-4'-nitrobenzamidine (3)

[0108] p-Anisidine (1.0 g, 8.1 mmol) was dissolved in anhydrous pyridine (15 ml), 4-nitrobenzoyl chloride (1.5 g, 8.1 mmol) was added. The reaction mixture was allowed to stand at room temperature for 16 hrs. The reaction mixture was poured into water and the precipitate was collected with filtrate under vacuum pressure and washed with 5% sodium bicarbonate (2x10 ml). The product 3 was used in the next step without further purification. 

1H NMR (300 MHz, DMSO-d$_6$) δ: 10.46 (s, 1H, NH), 8.37 (d, J=5.5 Hz, 2H, H-3',5'), 8.17 (d, J=6.3 Hz, 2H, H-2',6'), 7.48 (d, J=6.6 Hz, 2H), 6.97 (d, J=6.5 Hz, 2H), 3.75 (s, 3H, MeO).

(b) 4-Methoxy-4'-nitrothiobenzamidine (4)

[0109] A mixture of 4-methoxy-4'-nitrothiobenzamidine (1.0 g, 3.7 mmol) and Lawesson's reagent (0.89 g, 2.2 mmol, 0.6 equiv.) in chlorobenzene (15 ml) was heated to reflux for 4 hrs. The solvent was evaporated and the residue was purified with flash column (hexane/ethyl acetate=4:1) to give 820 mg (77.4%) of the product 4 as orange color solid. 

1H NMR (300 MHz, DMSO-d$_6$) δ: 8.29 (d, 2H, H-3',5'), 8.00 (d, J=8.5 Hz, 2H, H-2',6'), 7.76 (d, 2H), 7.03 (d, J=8.4 Hz, 2H), 3.808.37 (d, J=5.5 Hz, 2H, H-3',5'), 8.17 (d, J=6.3 Hz, 2H, H-2',6'), 7.48 (d, J=6.6 Hz, 2H), 6.97 (d, J=6.5 Hz, 2H), 3.75 (s, 3H, MeO), 3.1 (s, 3H, NMe$_2$).

(c) 6-Methoxy-2-(4-nitrophenyl)benzothiazole (5)

[0110] A mixture of 4-methoxy-4'-nitrothiobenzamidines (1.74 mmol) was wetted with a little ethanol (0.5 ml), and 30% aqueous sodium hydroxide solution (556 mg, 13.9 mmol, 8 equiv.) was added. The mixture was diluted with water to provide a final solution/suspension of 10% aqueous sodium hydroxide. Aliquots of this mixture were added at 1 min intervals to a stirred solution of potassium ferricyanide (2.29 g, 6.9 mmol, 4 equiv.) in water (5 ml) at 80-90°C. The reaction mixture was heated for a further 0.5 hr and then allowed to cool. The precipitate was collected by filtration under vacuum pressure and washed with water, purified with flash column (hexane/ethyl acetate=4:1) to give 130 mg (26%) of the product 5.

[0111] 1H NMR (300 MHz, Acetone-d$_6$) δ: 8.45 (m, 4H), 8.07 (d, J=8.5 Hz, 1H, H-4), 7.69 (s, 1H, H-7), 7.22 (d, J=9.0 Hz, 1H, H-5), 3.90 (s, 3H, MeO)

(d) 6-Methoxy-2-(4-aminophenyl)benzothiazole (6)

[0112] A mixture of the 6-methoxy-2-(4-nitropheny)benzothiazoles (22 mg, 0.077 mmol) and tin(II) chloride dihydrate (132 mg, 0.45 mmol) in boiling ethanol was stirred under nitrogen for 4 hrs. Ethanol was evaporated and the residue was dissolved in ethyl acetate (10 ml), washed with 1 N sodium hydroxide (2 ml) and water (5 ml), and dried over MgSO$_4$. Evaporation of the solvent gave 19 mg (97%) of the product 6 as yellow solid.

(e) 6-Methoxy-2-(4-aminophenyl)benzothiazole (7) and 6-Methoxy-2-(4-dimethylaminophenyl)benzothiazole (8)

[0113] A mixture of 6-methoxy-2-(4-aminophenyl)benzothiazole (8 mg, 0.059 mmol), MeI (8.3 mg, 0.060 mmol), and K$_2$CO$_3$ (100 mg, 0.72 mmol) in DMSO anhydrous (0.5 ml) was heated at 100°C for 16 hrs. The reaction mixture was purified by reverse phase TLC (MeOH:H$_2$O=7:1) to give 2.0 mg (13.3%) of 6-methoxy-2-4-methylaminophenylbenzothiazole 7 and 6 mg (40%) of 6-methoxy-2-(4-dimethylaminophenyl)benzothiazole 8. 1H NMR of 7 (300 MHz, Acetone-d$_6$) δ: 7.85 (d, J=8.7 Hz, 2H, H-2',6'), 7.75 (dd, J=8.8 Hz, J=1.3 Hz, 1H, H-4), 7.49 (d, J=2.4 Hz, 1H, H-7), 7.01 (dd, J=8.8 Hz, J=2.4 Hz, H-5), 6.78 (d, J=7.6 Hz, 2H, H-3',5'), 3.84 (s, 3H, MeO), 2.91 (s, 3H, NMe$_2$), 1H NMR of 8 (300 MHz, Acetone-d$_6$) δ: 7.85 (d, J=8.7 Hz, 2H, H-2'), 7.75 (dd, J=8.8 Hz, J=1.3 Hz, 1H, H-4), 7.49 (d, J=2.4 Hz, 1H, H-7), 7.01 (dd, J=8.8 Hz, J=2.4 Hz, H-5), 6.78 (d, J=7.6 Hz, 2H, H-3',5'), 3.84 (s, 3H, MeO), 3.01 (s, 6H, NMe$_2$).

[0114] Following the same strategy as above, the other claimed 2-(4-aminophenyl)-benzothiazole derivatives may be synthesized by substituting the appropriate substituted aniline derivative (e.g. 2-, 3-, or 4-methylaniline) and the appropriate 4-nitro-benzoyl chloride derivative (e.g. 2- or 3-methyl-4-nitro-benzoyl chloride).

Example 4

Synthesis of BTA Derivatives without $R^7-R^{10}$ Substitution

[0115] Route 2: Example of the synthesis of BTA-0, -1, -2 compounds, which are representative of the group of BTA compounds without $R^7-R^{10}$ (Garmaise et al., “Anthelmintic Quaternary Salts. III. Benzothiazolium Salts” J. Med. Chem. 12:30-36 1969):

![Diagram](https://via.placeholder.com/150)
A solution of 4-nitrobenzoyl chloride (1.49 g, 8.0 mmol) in benzene (anhydrous, 10 mL) was added dropwise to 2-aminothiophenol (1.0 g, 8.0 mmol in 10 mL of benzene) at room temperature. The reaction mixture was allowed to stir for 16 h. The reaction was quenched with water (20 mL). The aqueous layer was separately and extracted with ethyl acetate (3x10 mL). The combined organic layers were dried and evaporated. The crude product was purified with flash column, (hexane:ethyl acetate=85:15) to give 1.5 g (73.2%) of product as light yellow solid.

(b) 2-(4-Aminophenyl)benzothiazole (20)

A mixture of 2-(4-nitrophenyl)benzothiazole (105 mg, 0.40 mmol) and tin(II) chloride dihydrate (205 mg, 0.91 mmol) in ethanol (20 mL) was refluxed under N₂ for 4 hrs. After removing ethanol by vacuum evaporation. The residue was dissolved into ethyl acetate (20 mL), and washed with NaOH solution (1N, 3x20 mL) and water (3x20 mL), dried and evaporated to dryness to give 102 mg (97%) of the product.

(c) 2-(4-Methylaminophenyl)benzothiazole (21) and 2-(4-dimethylaminophenyl)benzothiazole (23)

A mixture of 2-(4-aminophenyl)benzothiazole 20 (15 mg, 0.066 mmol), Mel (9.4 mg, 0.066 mg) and K₂CO₃ (135 mg, 0.81 mmol) in DMSO (anhydrous, 0.5 ml) was heated at 100°C for 16 hrs. The reaction mixture was purified by reverse phase TLC (MeOH:H₂O=6:1) to give 1.5 mg (10%) of 2-(4-methylaminophenyl)benzothiazole 21 and 2.5 mg (16%) of 2-(4-dimethylaminophenyl)benzothiazole 23.

(d) 2-(4-Dimethylaminophenyl)benzothiazole (23)

The mixture of 2-aminothiophenol 9 (0.5 g, 4.0 mmol) 4-dimethylaminobenzoic acid 22 (0.66 g, 4.0 mmol) and PPA (10 g) was heated to 220°C for 4 hrs. The reaction mixture was cooled to room temperature and poured into a solution of 10% potassium carbonate (–400 mL). The residue was collected by filtration under vacuum pressure to give 964 mg of the product 23, which was ca. 90% pure based on the ¹H NMR analysis. Recrystallization of 100 mg of 23 in MeOH gave 80 mg of the pure product.

Example 5

Synthesis of bis-2,2’-(4′-aminophenyl)-dibenzothiazole derivatives

Route 1: Following the general procedure for 6-MeO-BTA compounds described above but substituting benzidine for p-anisidine and using 16 equivalents of 4-nitrobenzoyl chloride results in the following compound:
Biological Examples

Example 6
Determination of Affinity for Aβ and Brain Uptake of Thioflavin Derivatives

Initial competitive binding studies using [1H]CG and synthetic Aβ(1-40) were conducted to determine if CG, ThS and ThT bound to the same site(s). It has been determined that ThS competed with [1H]CG for binding sites on Aβ (1-40), but ThT did not (see, e.g., FIG. 5). High specific activity [N-methyl-14C]BTA-1 (see Table 1) was then synthesized by methylation of BTA-0. Bindings studies were performed with [N-methyl-14C]BTA-1 and 200 nM Aβ(1-40) fibrils. The specific binding of [N-methyl-14C]BTA-1 was ~70%. FIG. 5 (see the right panel) shows competition curves for Aβ sites by ThT, BTA-0, BTA-1, and BTA-2 using the [N-methyl-14C]BTA-1 binding assay. The Kᵢ’s were: 3.0±0.8 nM for BTA-2; 9.6±1.8 nM for BTA-1; 100±16 nM for BTA-0; and 1900±510 nM for ThT. Not only is the quaternary amine of ThT not necessary for binding to Aβ fibrils, it appears to decrease binding affinity as well.

In Table 1 below are five different 14C-labeled BTA derivatives where their in vitro binding properties, log P values, and in vivo brain uptake and retention properties in mice have been determined.

**TABLE 1**

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<tr>
<th>Structure of 14C-Labeled BTA Compound</th>
<th>Kᵢ (nM) to Aβ fibrils</th>
<th>Mouse Brain Uptake @ 2 min (ID/g*kg)</th>
<th>Mouse Brain Uptake @ 30 min (ID/g*kg)</th>
<th>Ratio of Uptake @ 2 min/30 min</th>
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<tr>
<td>H₃C-N₃N₃S-N₃H₃</td>
<td>21 (est.)</td>
<td>0.32 ± 0.07</td>
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<td>H₃C-N₃N₃S-N₃H₃</td>
<td>3.9 (est.)</td>
<td>0.15 ± 0.06</td>
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TABLE 1-continued

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<tr>
<th>Structure of ¹¹C-Labeled BTA Compound</th>
<th>Kᵦ (nM) to Aβ Fibrils</th>
<th>Mouse Brain Uptake @ 2 min (% ID/g*kg)</th>
<th>Mouse Brain Uptake @ 30 min (% ID/g*kg)</th>
<th>Ratio of 2 min/30 min Values</th>
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<tr>
<td>H²¹⁴CO</td>
<td>30</td>
<td>1.9</td>
<td>0.00 ± 0.04</td>
<td>0.39 ± 0.05</td>
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<td>6⁻¹¹C₆H₅-OBTA-0</td>
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<tr>
<td>H₂CO</td>
<td>5.7</td>
<td>2.7</td>
<td>0.43 ± 0.11</td>
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<tr>
<td>H₂CO</td>
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The data shown in Table 1 are remarkable, particularly for the ¹¹C-labeled 6-MeO-BTA-1 and BTA-1 derivatives. These compounds displayed relatively high affinity for Aβ, with Kᵦ values <10 nM, and readily entered mouse brain with uptake values >10% ID/g*kg (or >10% ID/g for 30 g animals). Moreover, the 30 min brain radioactivity concentration values were less than 0.1% ID/g*kg, resulting in 2 min-to-30 min concentration ratios >4. Both of the N,N-dimethyl compounds cleared less rapidly from mouse brain tissue than the N-methyl derivatives. Likewise, the only primary amine currently testable, 6-MeO-BTA-0, showed poor brain clearance. This surprising and unexpected result supports the specific use of the secondary amine (e.g. —NHCH₃) as in vivo imaging agent.

**Example 7**

In Vivo PET Imaging Experiments in Baboons

Large amounts of high specific activity (>2000 Ci/mmol) ¹¹C-labeled BTA-1, 6-Me-BTA-1, and 6-MeO-BTA-1 were prepared for brain imaging studies in 20-30 kg anesthetized baboons using the Siemens/CTI HR+ tomograph in 3D data collection mode (nominal FWHM resolution 4.5 mm). Brain imaging studies were conducted following the intravenous injection of 3-5 mCi of radiotracer. Typical attenuation- and decay-corrected time-activity curves for a frontal cortex region of interest for each of the three compounds are shown in FIG. 6. It is noted that the absolute brain uptake of these 3 compounds in baboons is very similar to that in mice (i.e., about 0.47 to 0.39% ID/g*kg). However, the normal brain clearance rate of all three radiotracers is considerably slower in baboons compared to mice, with peak-to-60 min ratios in the range of 2.4 to 1.6 compared to ratios as high as 7.7 at 30 min in mice. The rank order of maximum brain uptake and clearance rate of the three compounds were also the same in mice and baboons. Brain uptake of the radiotracers did not appear to be blood flow-limited (FIG. 6, inset). Arterial blood samples in the baboons following the injection of all three compounds were obtained, and showed that their metabolic profiles were quite similar. Only highly polar metabolites that eluted near the void volume (4 mL) of the reverse-phase analytical HPLC column were observed in the plasma at all time points following injection, while the unmetabolized tracer eluted at about 20 mL. Typical amounts of unmetabolized injectate in plasma for all three compounds were about 90% at 2 min; 35% at 30 min; and 20% at 60 min.

**Example 8**

Staining Amyloid Deposits in Post-Mortem AD and Tg Mouse Brain

Postmortem brain tissue sections from AD brain and an 8 month old transgenic PS1/APP [explain what this model
is used to show mouse were stained with unlabeled BTA-1. The PS1/APP mouse model combines two human gene mutations known to cause Alzheimer’s disease in a doubly transgenic mouse which deposits Aβ fibrils in amyloid plaques in the brain beginning as early as 3 months of age. Typical fluorescence micrographs are shown in FIG. 8, and the staining of amyloid plaques by BTA-1 in both postmortem AD and PS1/APP brain tissue is clearly visible. Cerebrovascular amyloid also was brightly stained (FIG. 8, right). The other characteristic neuropathological hallmark of AD brain, neurofibrillary tangles (NFT), are more faintly stained by BTA-1 in AD brain (FIG. 8, left). NFT have not been observed in transgenic mouse models of amyloid deposition.

Example 9
In Vivo Labeling and Detection of Amyloid Deposits in Transgenic Mice

Three 17 month-old PS1/APP transgenic mice were injected intraperitoneally (ip) with a single dose of 10 mg/kg of BTA-1 in a solution of DMSO, propylene glycol, and pH 7.5 PBS (v/v/v 10/45/45). Twenty-four hours later, multi-photon fluorescence microscopy was employed to obtain high resolution images in the brains of living mice using a cranial window technique. Typical in vivo images of BTA-1 in a living PS1/APP mouse are shown in FIG. 9, and plaques and cerebrovascular amyloid are clearly distinguishable. The multi-photon microscopy studies demonstrate the in vivo specificity of BTA-1 for Aβ in living PS1/APP transgenic mice.

Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

We claim:
1. An amyloid binding compound of the following formula or a water soluble, non-toxic salt thereof:

wherein
Y is NR'R”; Z is S; R’ is H;
wherein R is selected from the group consisting of a lower alkyl group, (CH₃)₂OR’ (wherein n=1, 2, or 3), CF₃, CH₂–CH₂X, O–CH₂–CH₂X, CH₂–CH₂–CH₂X (wherein X=F, Cl, Br or I), R and (CH₂).R., (wherein n=2, 3, or 4) R, represents an optionally substituted phenyl group); or

wherein M is selected from the group consisting of Tc and Re;
R’ is selected from the group consisting of H, F, Cl, Br, 1, a lower alkyl group, (CH₃)₂OR’ (wherein n=1, 2, or 3), CF₃, CH₂–CH₂X, O–CH₂–CH₂X, CH₂–CH₂–CH₂X (wherein X=F, Cl, Br or I), CN, (C=O)–R’, NO₂, (C=O)N(R’), O(CO)R’, OR’, SR’, COOR’, R’NH, or (CH₂)₂NH. M is selected from the group consisting of N₂, N₃, CN, (C=O)R’ or R’, or a chelating group (with or without a chelated metal group) of the form W-L, wherein W is —(CH₃)ₙ, where n=2, 3, 4, or 5; and L is:

wherein R₂ is a chelating group (with or without a chelated metal group) of the form W-L, wherein W is —(CH₂)ₙ, where n=2, 3, 4, or 5; and L is:
(C=O)NR(R')₂, O(CO)R', OR', SR', COOR', Rpₘₚ, CR'₂=CR'₃=CR'₄=CR'₅=CR'₆=Rpₘₚ (wherein R' is H or a lower alkyl group and Rpₘₚ represents an optionally substituted phenyl group), and a tri-alkyl tin;

R⁻ is selected from the group consisting of H, F, Cl, Br, I, a lower alkyl group, (CH₃)₂OR' (wherein n=1, 2, or 3), CF₃, CH₃CH₂X, O=CH₂=OCH₂X, CH₂−CH₂X, O=CH₂−CH₂X, CH₂−CH₂X (wherein X=F, Cl, Br or I), CN, (C=O)−R', N(R')₂, NO₂, (C=O)N(R')₂, O(CO)R', OR', SR', COOR', Rpₘₚ, CR'₂=CR'₃=CR'₄=CR'₅=CR'₆=Rpₘₚ (wherein R' is H or a lower alkyl group and Rpₘₚ represents an optionally substituted phenyl group), and a tri-alkyl tin;

Rₕ is selected from the group consisting of H, F, Cl, Br, I, ethyl, propyl, butyl, (CH₃)₂OR' (wherein n=1, 2, or 3), CF₃, CH₃−CH₂X, O=CH₂−CH₂X, CH₂−CH₂X, O=CH₂−CH₂X, CH₂−CH₂X (wherein X=F, Cl, Br or I), CN, (C=O)−R', N(R')₂, NO₂, (C=O)N(R')₂, O(CO)R', OR', SR', COOR', Rpₘₚ, CR'₂=CR'₃=CR'₄=CR'₅=CR'₆=Rpₘₚ (wherein R' is H or a lower alkyl group and Rpₘₚ represents an optionally substituted phenyl group), and a tri-alkyl tin;

R₀ is selected from the group consisting of H, F, Cl, Br, I, a lower alkyl group, (CH₃)₂OR' (wherein n=1, 2, or 3), CF₃, CH₃−CH₂X, O=CH₂−CH₂X, CH₂−CH₂X, O=CH₂−CH₂X, CH₂−CH₂X (wherein X=F, Cl, Br or I), CN, (C=O)−R', N(R')₂, NO₂, (C=O)N(R')₂, O(CO)R', OR', SR', COOR', Rpₘₚ, CR'₂=CR'₃=CR'₄=CR'₅=CR'₆=Rpₘₚ (wherein R' is H or a lower alkyl group and Rpₘₚ represents an optionally substituted phenyl group), and a tri-alkyl tin;

R₁₀ is selected from the group consisting of H, F, Cl, Br, I, a lower alkyl group, (CH₃)₂OR' (wherein n=1, 2, or 3), CF₃, CH₃−CH₂X, O=CH₂−CH₂X, CH₂−CH₂X, O=CH₂−CH₂X, CH₂−CH₂X (wherein X=F, Cl, Br or I), CN, (C=O)−R', N(R')₂, NO₂, (C=O)N(R')₂, O(CO)R', OR', SR', COOR', Rpₘₚ, CR'₂=CR'₃=CR'₄=CR'₅=CR'₆=Rpₘₚ (wherein R' is H or a lower alkyl group and Rpₘₚ represents an optionally substituted phenyl group), and a tri-alkyl tin;

or one of R²⁻R₁₀ is a chelating group (with or without a chelated metal group) of the form W-L⁻ or V-W⁻, wherein V is selected from the group consisting of −COO−, −CO−, −CH₂O− and −CH₂NH−; W is −(CH₂)ₙ where n=0, 1, 2, 3, 4, or 5; and L is:

wherein M is selected from the group consisting of Tc and Re.

3. The compound of claim 1, wherein R²⁻=CH₃, R³⁻=H, R⁸⁻=OH and R⁶⁻R¹⁰ are H.

4. The compound of claim 1, wherein R²⁻=CH₃ and R⁸⁻ is selected from the group consisting of CN, CH₃, OH, OCH₃ and NH₃.
5. The compound of claim 4, wherein R³-R⁷ and R⁹-R¹⁰ are H.

6. The compound of claim any one of claims 1-5, wherein the compound binds to Aβ with a dissociation constant (K_d) between 0.0001 and 10.0 μM when measured by binding to synthetic Aβ peptide or Alzheimer’s Disease brain tissue.