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Discovery and characterization of novel indole and 7-azaindole derivatives as inhibitors of β-amyloid-42 aggregation for the treatment of Alzheimer's disease

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ABSTRACT

The aggregation of amyloid-β peptides into cytotoxic oligomeric and fibrillary aggregates is believed to be one of the major pathological events in Alzheimer disease. Here we report the design and synthesis of a novel series of indole and 7-azaindole derivatives containing, nitrile, piperidine and N-methyl-piperidine substituents at the 3-position to prevent the pathological self-assembly of amyloid-β. We have further demonstrated that substitution of the azaindole and indole derivatives at the 3 positions is required to obtain compounds with improved physicochemical properties to allow brain penetration.

Alzheimer's disease (AD) is a form of senile dementia, characterized by a progressive loss of memory and cognitive function[1-3]. It has been hypothesized that formation of β-amyloid (Aβ) plaques is key to the development and progression of the disease. Present pharmacotherapies with known anti-cholinesterase activity, such as Aricept and Exelon, are only helpful to alleviate some of the symptoms for a limited time period[4]. These agents act to stabilize the remaining neuronal networks and prolong neuronal function until their therapeutic effect diminishes and drug tolerance occurs. The marginal benefits from these therapies emphasize the urgent need to develop alternative and effective disease-modifying agents[5]. Several trends have been emerging using small molecules to target various AD pathological routes such as the amyloidogenic secretases (β/γ- secretase)[6-7], amyloid-β aggregation[8-14], tau phosphorylation and fibrillation[15-17] and metal-ion redox/reactive oxygen species (ROS)[18-22].

Of those, Aβ is one of the most promising targets for the development of new therapies as the substantial data derived from genetics, animal modeling, and biochemical studies support the idea that Aβ, the major component of senile plaques, plays a central role in AD pathophysiology[23-24]. Thus, we have initiated a program aiming to design novel non dye compounds for the inhibition of Aβ aggregation for AD therapeutics. We have previously reported small molecule inhibitors of Aβ based on our rational design, *i.e.* 3-aminopyrazole[24] and 2,6-disubstituted pyridine derivatives[25] which can interact via a donor-acceptor-donor (DAD) hydrogen bond pattern complementary to that of the β-sheet of Aβ[22-24]. However, compounds following this design displayed low metabolic stability and poor PK properties[25]. To overcome these issues, we sought to evaluate whether replacing the 2,6-disubstituted pyridine moiety with 6-amino-substituted indole and 7-azaindole moieties (Fig. 1) would improve metabolic stability and PK properties while maintaining their inhibition of Aβ aggregation properties.

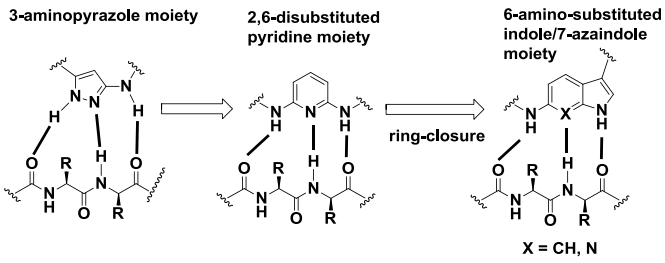

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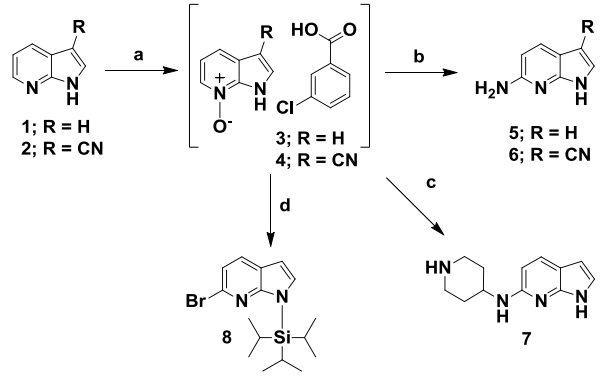
Figure 1. Possible hydrogen bond interactions of 3-aminopyrazole, 2,6-disubstituted pyridine and 6-amino-indole/7-azaindole derivatives with the β-sheet conformation of Aβ.

In order to synthesize the novel compounds containing indole or 7-azaindole moieties, it was necessary to prepare the required building blocks.

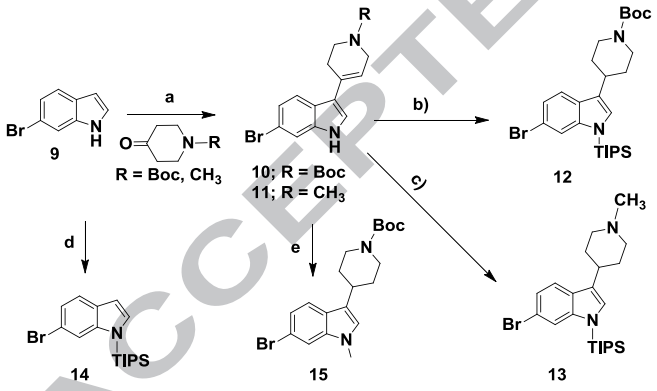
Thus, suitable 7-azaindole or indole derivatives containing either bromo- or amino substituents were synthesized. The reaction of commercially available 7-azaindole **1** and 7-azaindole-carbonitrile **2** with m-CPBA in diethyl ether gave the corresponding salts **3** and **4**. The suspensions of **3** or **4** in acetonitrile were treated with dimethyl sulfate under reflux followed by quenching with a concentrated ammonia solution in methanol[33] to afford the corresponding amines **5** and **6** (Scheme 1).



Scheme 1. Reagents and conditions: (a) m-CPBA, Et_2O, rt, 18 h, 86-96%; (b) (i) dimethylsulfate, CH_3CN, 70 °C, 8 h; (ii) 7M NH_3/CH_3OH, 60 °C, 48 h, 41-54%; (c) (i) dimethylsulfate, CH_3CN, 70 °C, 16 h; (ii) 1-Boc-4-amino-piperidine, CH_3OH, 50-60 °C, 3d, 36-45%; (iii) 2M HCl/Et_2O, rt,; (d) (i) K_2CO_3, H_2O, rt, 91%; (ii) HMDS, toluene, benzoyl bromide, rt, 1 h, 65%; (iii) NaOH, CH_3OH, rt, 16 h, 60%.

The synthesis of **7** was achieved from **3** as described for **5**, employing *tert*.-butyl 4-aminopiperidine-1-carboxylate followed by acid cleavage of the *tert*-butyloxycarbonyl (Boc) protecting group [33] The synthesis of the N^1-triisoproylsilyl (TIPS) protected 7-azaindole derivative **8** containing a 6-bromo-substituent was achieved by treating **3** with benzoyl bromide in the presence of hexamethyldisiliazane (HMDS), followed by saponification of the benzoyl group with sodium hydroxide in methanol, followed by TIPS-protection of the N^1-position of the 7-azaindole moiety to provide **8.**

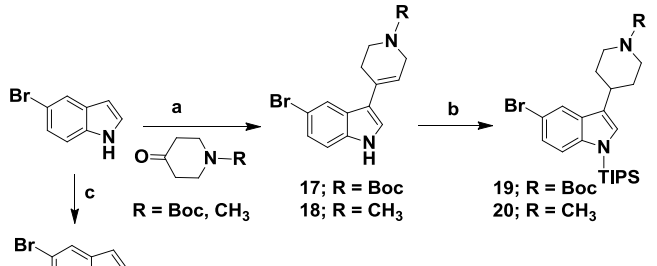
The corresponding 3-substituted indole and azaindole building blocks **12**, **13**, **14** and **15** containing a 6-bromo-substituent were prepared according to Scheme 2.



Scheme 2. Reagents and conditions: (a) 25% $NaOCH_3/CH_3OH$, 80°C, 48 h, 52-91%; (b) (i) H_2, PtO_2, TEA, CH_3OH, rt, 18 h, 63%; NaH, THF, TIPS-Cl, rt, 30 min, 91%; (c) (i) NaH, THF, TIPS-Cl, rt, 30 min, 65%; (ii) H_2, PtO_2, TEA, EtOAc, rt, 18 h, 85%; (d) NaH, THF, TIPS-Cl, 30 min, rt, 99%; (e) H_2, PtO_2, TEA, CH_3OH, rt, 18 h, 63%; (ii) NaH, THF/CH_3I, 30 min, 94%

The synthesis of **14** was achieved from **9** by protecting the N^1-position of 6-bromo-indole **9** with TIPS-Cl in the presence of sodium hydride as base to afford **14.** The condensation reaction of 6-bromo-indole **9** with *tert*.-butyl 4-oxopiperidine-1-carboxylate or 1-methylpiperidin-4-one in the presence of sodium methoxide in methanol yielded the corresponding 3-substituted 6-bromo-indole derivatives **10** and **11**. Reduction of the double in **10** with platinum(IV) oxide, followed by TIPS-protection of the N^1-position provided the corresponding piperidine-derivative **12.** TIPS-protection of the N^1–position of compound **11** followed by double bond reduction yielded **13.** N^1-methylation of **10** with methyl iodide followed by reduction of double bond afforded compound **15**.

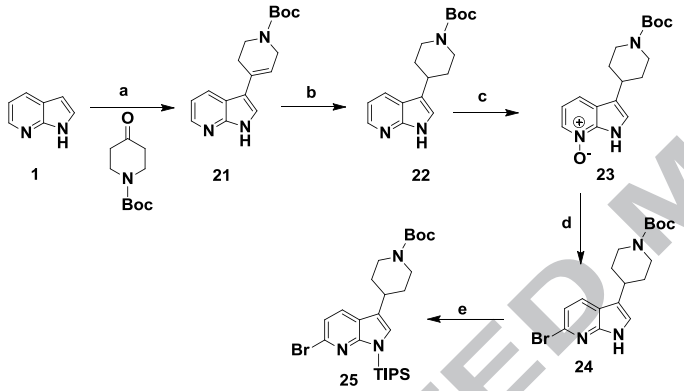
To test positional effects on indole and 7-azaindole core structures, the indole building blocks **19** and **20** containing a 5-bromo-substituent were prepared (Scheme 3).



Scheme 3. Reagents and conditions: (a) 25% $NaOCH_3/CH_3OH$, 80°C, 48 h, 73-86%; (b) (i) NaH, THF, rt, 10 min; (ii) H_2, PtO_2, TEA, CH_3OH, rt, 16 h, 30-50%. (iii) TIPS-Cl, rt, 1-2 h, 65-70%

Commercially available 5-bromo-indole was protected with TIPS to yield **16**. The 5-bromo-indole was reacted with *tert*.-butyl 4-oxopiperidine-1-carboxylate or 1-methylpiperidin-4-one as described to afford the corresponding 3-substituted 5-bromo-indole derivatives **17** and **18**. Reduction of the 1,2,3,6-tetrahydropyridin moiety in **17** and **18** with platinum(IV) oxide, followed by TIPS-protection of the N^1-position provided the corresponding piperidine-derivatives **19** and **20**.

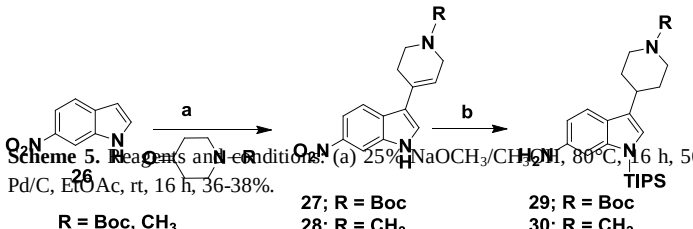
The corresponding 6-bromo-7-azaindole building block containing a piperidine substituent at the 3-position was prepared from **1** (Scheme 4).



Scheme 4. (a) 25% $NaOCH_3/CH_3OH$, 80°C, 2 h 30 min, 67%; (b) (i) H_2, Pd/C, CH_3OH, rt, 48 h, 91%; (c) m-CPBA, CH_2Cl_2, rt, 12 h, 74%; (d) (i) HMDS, toluene, benzoyl bromide, rt, 12 h; (ii) NaOH, CH_3OH, rt, 2 h, 40%; (e) (i) NaH, DMF, rt, 1 h; (ii) TIPSCl, rt, 19 h, 71%.

The condensation reaction of **1** with *tert*.-butyl 4-oxopiperidine-1-carboxylate as described yielded the corresponding 3-substituted 7-azaindole derivative **21**. Reduction of the double bond was carried out with palladium on carbon, under the hydrogen gas pressure to give corresponding reduced product **22.** The N-oxide derivative **23** was prepared by treatment with m-CPBA. Treatment of **23** with benzoyl bromide in the presence of HMDS, followed by saponification of the benzoyl group provided **24.** TIPS-protection of the N^1-position of **24** afforded the desired building block **25**.

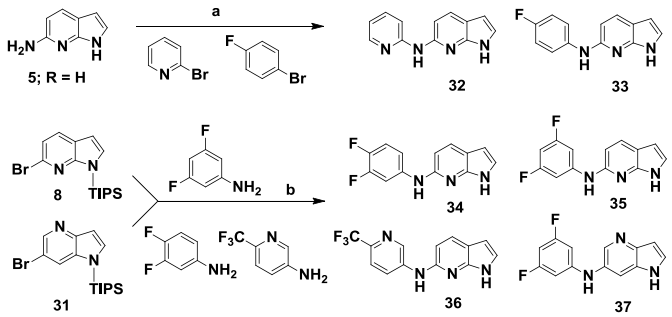
The corresponding 6-amino-indole building blocks containing a piperidine substituent at the 3-position were prepared from 6-nitro indole **26** (Scheme 5). Treatment of **26** with *tert*.-butyl 4-oxopiperidine-1-carboxylate or 1-methylpiperidine-4-one as described yielded the corresponding 3-substituted 7-indole derivatives **27** and **28**. TIPS-protection of **27** and **27** followed by hydrogenation with palladium on carbon afforded building blocks **29** and **30**.



Scheme 5. Reagents and conditions: (a) 25% $NaOCH_3/CH_3OH$, 80°C, 16 h, 56-72%; (b) (i) NaH, THF, rt, 10 min; (ii) TIPSCl, rt, 1 h, 46-50%; (iii) H_2, Pd/C, EtOAc, rt, 16 h, 36-38%.

A small library of compounds having a 7-azaindole moiety linked to a substituted phenyl or pyridine moiety via an amine linker (Scheme 6) was prepared first to get an idea about the structural motifs necessary to retain some of the inhibition of aggregation properties described for our previous compounds[24, 25]. We employed Buchwald reaction conditions for C-N bond formation. A

highly active palladium(0) catalyst was generated from palladium(II) acetate ($Pd(OAc)_2$) and 2-dicyclohexyl-phosphino-2′,4′,6′-triisopropyl-biphenyl (XPhos)[29-31] to enable the coupling of the bromo- and amino building blocks (Scheme 6).



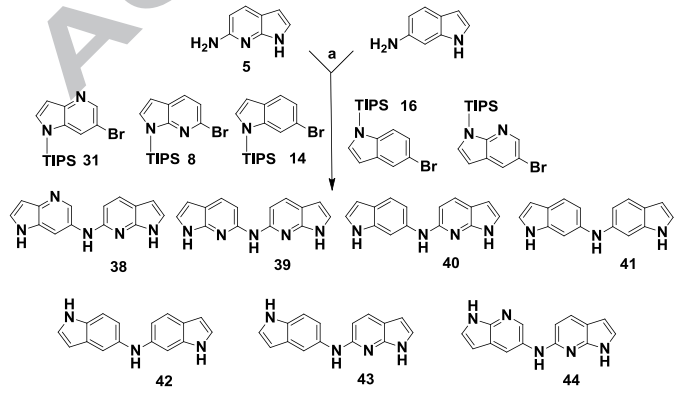
Library- 1: *Compound structures of azaindole derivatives*

Scheme 6. Reagents and conditions: (a) $Pd(OAc)_2$, XPhos, NaOtBu, 2-bromopyridine or 1-bromo-4-fluorobenzene, dioxane, 110°C, 2 h, 39-94%. (b) (i) $Pd(OAc)_2$, XPhos, 3,5-difluoroaniline, NaOtBu, dioxane, 110°C, 2 h, 50% or $Pd(OAc)_2$, XPhos, 3,4-difluoroaniline or 3,5-difluoroaniline or 6-(trifluoromethyl)pyridin-3-amine, K_2CO_3, t-BuOH, 110°C, 3 h, 77-91%; (ii) 1 M TBAF/THF, CH_3CN, rt, 1 h; (iii) 1 M HCl/H_2O, 66-81%.

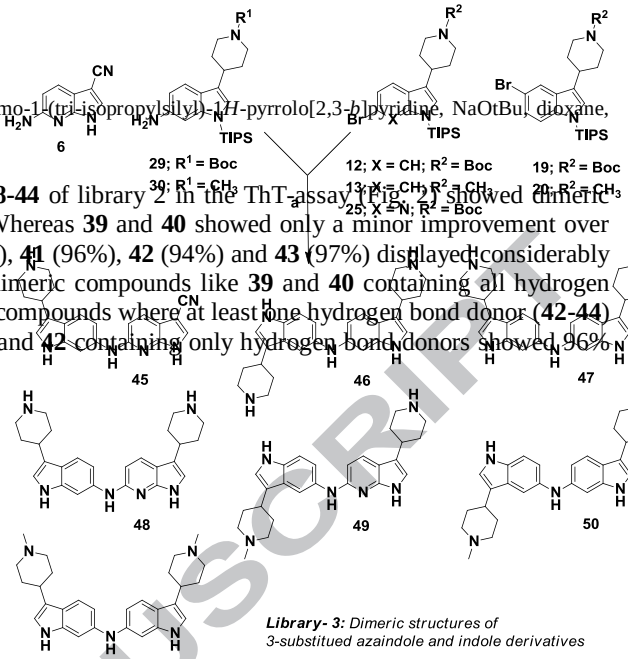
The reaction of 6-amino-azaindole **5** with 2-bromo-pyridine or 1-bromo-4-fluorobenzene afforded the final compounds **32** and **33**. TIPS-protected building blocks **8** and commercially available 6-bromo-1-(triisopropylsilyl)-1*H*-pyrrolo[3,2-*b*]pyridine **31** were reacted under Buchwald conditions with aniline derivatives to form the intermediate palladium coupling products, which were treated with tetra-butylammonium fluoride (TBAF) to cleave the TIPS-protecting group. The final compounds **32-37** were isolated after acid treatment as HCl-salts.

In our screening cascade of small molecule inhibitors of Aβ peptide, the thioflavin-T (ThT) fluorescence assay[27] using crude $A\beta_{1-42}$ peptide film at 33 μM and compounds at 330 μM was employed as a primary screen. The data in Figure 2 indicated compounds **32-37** of library 1 displayed inhibition of Aβ aggregation in the range of 52% (**33**) to 71% (**32**). Exchange of a phenyl or pyridine ring in **32-37** with piperdine to obtain additional hydrogen bond donor interactions, did not result in an improved compound as **7** displayed only 48% inhibition of Aβ aggregation. However, this could be explained by the lack of additional aromatic π-π interactions of compound **7** with the Aβ peptide. Compounds **35** and **37** showed inhibition of Aβ aggregation of 60% and 52%, respectively. This indicated shifting the hydrogen bond acceptor from 7-azaindole-core (**35**) to a 4-azaindole-core (**37**) had little effect on inhibition Aβ aggregation. Due to their small size, compounds **32-37** appeared not to be able to pick-up additional π-π interactions to compensate for the reduction in DAD interactions. Thus, we identified a new scaffold containing an azaindole/indole core, but the inhibition of Aβ aggregation was lower compared to the best compounds containing the 3-aminopyrazole[24] or 2,6-disbstituted pyridine moieties[25] we reported earlier.

In order to increase the inhibition of Aβ aggregation properties and further diversify the scaffold, we envisaged inhibition of Aβ aggregation would be improved by dimeric compounds (**38-44**), which can offer additional hydrogen bond donor and aromatic π-π interactions with the Aβ peptide. The synthesis of dimeric compounds **38-44** (Scheme 7) was achieved by Buchwald Pd-coupling reaction conditions[31] of amino building block **5** and commercially available 6-aminoindole with bromo building blocks **8**, **14**, **16**, **31**, and commercially available 5-bromo-1-(triisopropylsilyl)-1*H*-pyrrolo[2,3-*b*]pyridine, as the first step. Cleavage of the TIPS-protecting group with TBAF followed by acid treatment afforded dimeric compounds **38-44**. The synthesis of **38** was achieved by pd-coupling reaction of 7-aminoazaindole **5** and commercially available corresponding bromo building block **31**. Testing the inhibition of Aβ aggregation properties of **38** (98%) in ThT, displayed pronounced activity when compared to compound **32.** The encouraging results of **38** from ThT assay, we then expanded the library of dimeric compounds **38-44.**

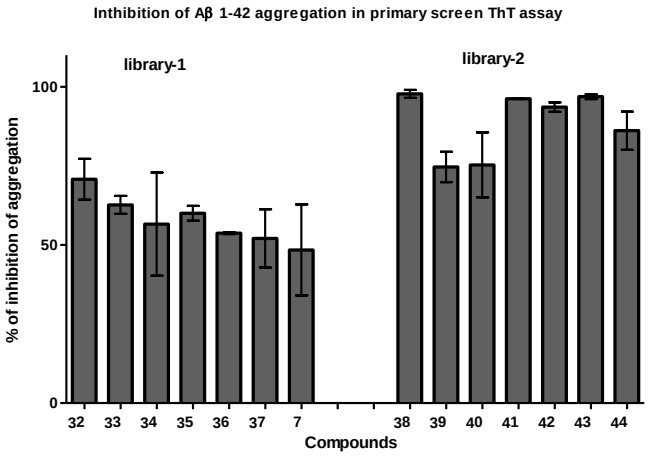


Library- 2: *Dimeric structures of azaindole and indole derivatives*



Scheme 7. Reagents and conditions: (a) (i) $Pd(OAc)_2$, XPhos, **8**, **14**, **16**, **31**, 5-bromo-1-(tri-isopropylsilyl)-1*H*-pyrrolo[2,3-*b*]pyridine, NaOtBu, dioxane, 110°C, 2 h, 6-30%; (ii) 1M TBAF/THF, CH_3CN, rt, 1 h; (iii) 1 M HCl/H_2O, 48-84%.

Testing the inhibition of Aβ aggregation properties of compound **38-44** of library 2 in the ThT-assay (Fig. 2) showed dimeric compounds displayed inhibition of Aβ aggregation between 75-98%. Whereas **39** and **40** showed only a minor improvement over **32**, i.e. 71% vs. 75% inhibition of Aβ aggregation, compounds **44** (86%), **41** (96%), **42** (94%) and **43** (97%) displayed considerably better inhibition of Aβ aggregation as **32**. These data suggested that dimeric compounds like **39** and **40** containing all hydrogen bond donors and acceptors on one side of the molecule were inferior to compounds where at least one hydrogen bond donor (**42-44**) or acceptor (**38**) was on the opposite site. Interestingly, compounds **41** and **42** containing only hydrogen bond donors showed 96% and 94% inhibition of Aβ aggregation, respectively.



Library- 3: *Dimeric structures of 3-substitued azaindole and indole derivatives*

Figure 2: $A\beta_{1-42}$ lyophilized powder was reconstituted in hexafluoroisopropanol to 1 mM to prepare the $A\beta_{1-42}$ peptide film. $A\beta_{1-42}$ inhibition of aggregation measured by a standard ThT assay at 1:10 $A\beta_{1-42}$ to compound molar ratio (33:330 μM). The data are expressed as mean of two independent experiments. Data are expressed as percentage (mean ± standard deviation) of control conditions: $A\beta_{1-42}$ aggregation with DMSO only.

Taken together the results of library 1 & 2, aromatic π-π interactions and orientation hydrogen bond donor and acceptors are quite important. In line with previous results of our 2,6-disubstitued pyridine derivatives[25], 3 hydrogen bond donors in the correct arrangement were required to achieve >80% inhibition of Aβ aggregation in the ThT-assay. In contrast to the 2,6-disubstitued pyridine derivatives, ThT-data for compounds **38-44** suggested the hydrogen bond acceptors were not required for inhibition of Aβ aggregation. Compound **38** was selected as representative molecule having a dimeric structure to assess its *in vitro* stability. However, **38** displayed poor metabolic stability in human liver microsomes with a half-life of only 11 minutes. It was also found that indole-3 position was susceptible for oxidation and causing for poor metabolic stability in a metabolic identification study (data not shown).

In order to block the potential metabolic weak spot at the 3 position of the indole/7-azaindole moieties, nitrile, piperidine and N-methyl piperidine substituents were introduced with the aim to maintain the inhibition of Aβ aggregation properties of **38-44** but to improve metabolic stability and solubility. The synthesis of dimeric compounds **45-51** having nitrile, piperidine and N-methyl piperidine substituents was achieved by Buchwald Pd-coupling reaction conditions[29-31] of amino building blocks **6** , **29** and **30** with bromo building blocks **12**, **13**, **19**, **20** and **25** as the first step (Scheme 8) to yield corresponding TIPS protected Pd-coupling derivatives. The cleavage of the TIPS-protecting group with TBAF or polymers supported fluorine followed by acid treatment afforded compounds **45-51** of library 3 (Scheme 8).

The ThT-assay data of compounds **45-51** of library 3 in Figure 3 indicated >90% inhibition of Aβ aggregation for all compounds. Thus, no preference was observed for either of the nitrile, piperidine and N-methyl piperidine substituents or the orientation of the indole/7-azaindole core structures, i.e. 6,6-substitution (**45**, **47**, **48**, **51**) versus 5,6 substitution (**46**, **49**, **50**). The >90% inhibition of Aβ aggregation of compounds **45-51** could be rationalized by an additional hydrogen bond donors. To test our hypothesis for improved metabolic stability in human liver microsomes, compounds **45-51** were characterized *in vitro* to assess their solubility, metabolic stability, permeability and if they are P-gp substrates (Table 1). The solubility of **45**, **46** and **48** in PBS (pH 7.4) was between 153-196 μM and we assumed compounds **47**, **49-51** would show a similar behavior. Incubation with human liver microsomes showed that compounds **45-51**, were quite stable (89-100% parent remaining after 1 hour), which was a marked improvement compared to compound **38**.

Scheme 8. Reagents and conditions: (a) (i) $Pd(OAc)_2$, XPhos, NaOtBu, dioxane, 110°C, 2 h, or $Pd(OAc)_2$, XPhos, K_2CO_3, t-BuOH, 110°C, 3 h, **6**, **29**, **30**, **12**, **13**, **25**, **19**, **20**, 30-79%; (ii) 1M TBAF/THF, CH_3CN, rt, 1 h or polymer-supported fluorine, THF, rt, 16 h, 60-92%; (iii) 2 M HCl/Et_2O, rt, 16 h, 56-99%.

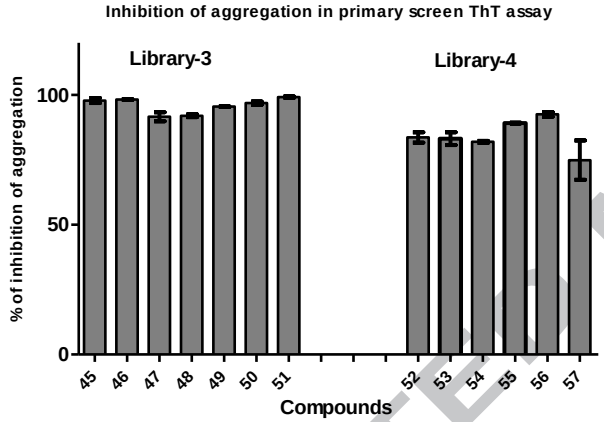
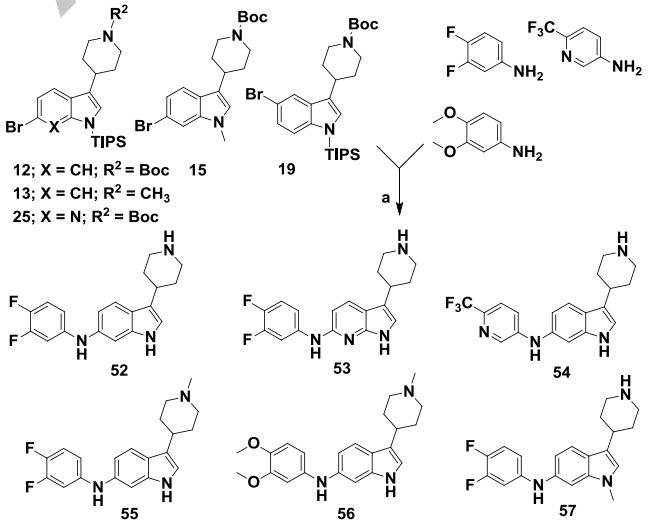


Figure 3: $A\beta_{1-42}$ lyophilized powder was reconstituted in hexafluoroisopropanol to 1 mM to prepare the $A\beta_{1-42}$ peptide film. $A\beta_{1-42}$ inhibition of aggregation measured by a standard ThT assay at 1:10 $A\beta_{1-42}$ to compound molar ratio (33:330 μM).The data are expressed as mean of two independent experiments Data are expressed as percentage (mean ± standard deviation) of control conditions: $A\beta_{1-42}$ aggregation with DMSO only.

However, permeability assessment u sing a sub-clone of the Caco-2 cell line showed rather low apical-to-basolateral (A->B) permeability (0.02-0.5 x 10^{-6} cm/s) for compounds **45-51**, most likely caused by the multiple hydrogen bond donors (three to five) and the presence of two aliphatic amine moieties. In addition, compounds **45**, **50** and **51** were P-gp substrates with efflux ratios of 21-100. Thus, no plasma or brain uptake of **46** after oral administration of 10 mg/kg to male Swiss mice could be detected (data not shown).

Thus, it was necessary to reduce the number of hydrogen bond donors and aliphatic amine moieties. The aim was to obtain compounds able to inhibit Aβ aggregation comparable to **45-51**, with improved permeability and PK properties. As there was no marked difference in inhibition of Aβ aggregation of library 2 (**38-41)** and library 3 **(45-51**) compounds, we envisaged combining the right-hand side scaffold of library 3 (**45-51**) *i.e.* 3-piperidine-substituted indole- or 7-azaindole moiety with the substituted phenyl-derivatives used for library 1 (**32-37**).



Library- 4: *Compound structures 3-substitued azaindole and indole derivatives*

Scheme 9. Reagents and conditions: (a) (i) $Pd(OAc)_2$, XPhos, NaOtBu, dioxane, 110°C, 2 h, or $Pd(OAc)_2$, XPhos, K_2CO_3, t-BuOH, 110°C, 3 h, 3,4-difluroaniline, 3, 4-dimethoxyaniline, 6-(trifluoromethyl)pyridin-3-amine, **12**, **13**, **19**, **25**, 47-75%; (ii) 1 M TBAF/THF, CH_3CN, rt, 1 h or polymer-supported fluorine, THF, rt, 16 h, 68-80%; (iii) 2 M HCl/Et_2O, rt, 16 h, 78-83%.

The synthesis of compounds **52-57** having a lower molecular weight, two to three hydrogen bond donors and only one aliphatic amine was achieved by Buchwald Pd-coupling reaction conditions[32-34] of 3,4-difluro- 3, 4-dimethoxyaniline and 6-(trifluoromethyl)pyridin-3-amine with bromo building blocks **12**, **13**, **19**, and **25** as the first step (Scheme 9, and see compound **57** synthesis detail in supplementary information). Cleavage of the TIPS-protecting group with TBAF followed by acid treatment afforded compounds **52-57** of library 4. Compounds **52-57** displayed inhibition of Aβ aggregation of 81-93% in the ThT-assay (Fig. 3). Thus, it was possible to reduce the number of hydrogen bond donors to two or three and aliphatic amines to one but maintaining inhibition of Aβ aggregation similar to compounds **52-57**.

As compounds **45-57** displayed >80% inhibition of Aβ aggregation in the ThT-assay under screening conditions, it was necessary to determine IC_{50} values of selected compounds to see differences. Subsequently, we assessed the IC_{50} values of compounds **38, 45, 46, 48, 50, 52, 53, 54, 55, 56, 57** in the ThT assay with Congo Red and Curcumin as reference standards. Compounds **38**, **45**, **46**, **48**, **50**, **52**, **53**, **54** and **56** were more potent than **55** and **57** (Fig. 4). Thus, reducing the number of hydrogen bond donors from three to two (**55**, **57**) resulted in a drop of potency. Compounds **45** and **50** (library 3) displayed inhibition of Aβ aggregation properties comparable to Curcumin with IC_{50} of 17.8 μM and 15.3 μM, respectively. Though compound **56** contained only two hydrogen bond donors, inhibition of Aβ aggregation was comparable to compounds **38**, **46**, **48**, **52** and **54**, i.e. IC_{50}< 30 μM, by introducing the 3,4-dimethoxy-phenyl moiety. Congo Red remained the most potent compound in the ThT-assay.

In vitro characterization (Table 1) showed compounds **52-57** have a solubility in PBS (pH 7.4) of 154-200 μM, are metabolically stable when incubated with human (74-95% parent remaining after 1 hour) and mouse liver microsomes (70-85% parent remaining after 1 hour). Only compound **55** was less stable in mouse liver microsomes (30% parent remaining after 1 hour). Compounds **52-57** displayed good apical-to-basolateral (A->B) permeability (3-24 x 10^{-6} cm/s) and were not P-gp substrates (efflux ratio <1), except compound **54** (efflux ratio of 33). Thus, compounds **52-54**, and **56** combined inhibition of Aβ aggregation properties of IC_{50}< 30 μM, comparable to potent 2,6-disubstituted pyridine derivatives[25], with improved physicochemical properties.

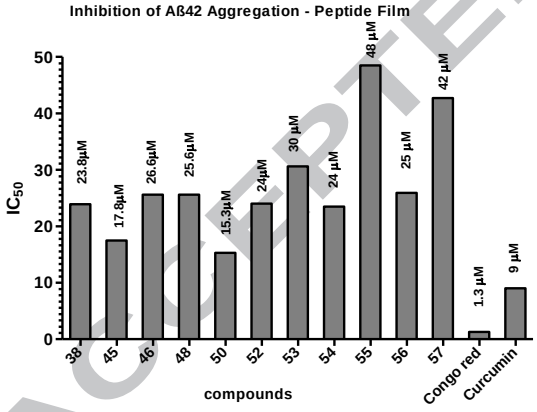


Figure **4.** IC_{50} determination by ThT assay using $A\beta_{1-42}$ peptide film. The concentration of $A\beta_{1-42}$ peptide film was 33 μM. The test concentration for compounds **38**, **45**, **46**, **48**, **50**, **52**, **53**, **54**, **55**, **56**, **57**, Congo Red and Curcumin were 330 μM, 82.5 μM, 20.63 μM, 5.16 μM, 1.29 μM, 0.32 μM and 0.08 μM. The IC50 values were determined from the fluorescence values obtained. The IC_{50}-data (μM) are expressed as mean ± standard deviation and indicated on top of each graph.

Table 1: Summary of ADME results of compounds

	Solubility in PBS [μM][a]	Metabolic stability human [% parent][b]	Metabolic stability mouse [% parent][b]	A->B permeability [x 10^{-6} cm/sec]	Efflux ratio
45	196	100	85	0.1	>100
46	153	92	n.d.[c]	0.1	1
47	n.d.[c]	89	n.d.[c]	0.01	0
48	166	95	n.d.[c]	0.4	0.5
49	n.d.[c]	95	n.d.[c]	0.02	n.d.[c]
50	n.d.[c]	100	n.d.[c]	0.5	32
51	n.d.[c]	100	n.d.[c]	0.3	21
52	178	89	85	7.1	0.51
53	176	95	81	14.5	0.64
54	n.d.[c]	90	n.d.[c]	1.2	33
55	200	91	35	10.4	0.18
56	200	92	70	24	0.96
57	169	77	83	3	0.53

[a]Determined at pH 7.4 in phosphate buffered saline (PBS)
[b]Remaining parent compound after 1 hour of incubation at 37°C.
[c]Not determined (n.d.).

However, compounds **52**, **53** did not display good brain exposure after oral administration at 10 mg/kg to male Swiss mice (data not shown) despite having good permeability (>4 x 10^{-6} cm/s) and showing good metabolic stability (human and mice liver microsomes). Thus, it appeared compound **52**, **53** were still too polar with three hydrogen bond donors including one secondary amine. Reducing the number of hydrogen bond donors by N^1-alkalytion did improve brain uptake for **57** after oral administration at 10 mg/kg to male Swiss mice (data not shown). However, only a small amount of **57** (~200 ng/g) in the brain of male Swiss mice could be detected after 2 hours. In contrast, compounds **55** and **56** displayed significant brain uptake in a four time-point PK-assessment after oral administration at 10 mg/kg to male Swiss mice (Fig. 5) with a brain-to-plasma (B/P) exposure ratio >1 (Table 2). Reducing the number of hydrogen bond donors to two, and conversion of the secondary amine (piperidine moiety) to a tertiary amine (N-methyl piperidine moiety) was most likely the reason for the improved brain uptake. Interestingly, **55** exhibited high brain exposure despite of its reduced metabolic stability in mice liver microsomes

Table 2: PK parameter of **55** and **56** in male Swiss mice[a]

	55 (plasma)[b]	**55 (brain)[a]**	**56 (plasma)[a]**	**56 (brain)[a]**
$t_{1/2}$ (h)	2.66	2.74	1.85	ND
AUC (ng.h/mL or ng.h/g)0-last	711	39584	2495	4040
C_{max} (ng/mL or ng/g)	107	5833	549	642
B/P[b]	55.5		2.1	

[a]Administration at 10 mg/kg p.o.
[b]B/P = brain to plasma ratio, at C_{max} at 4h post p.o. dosing.

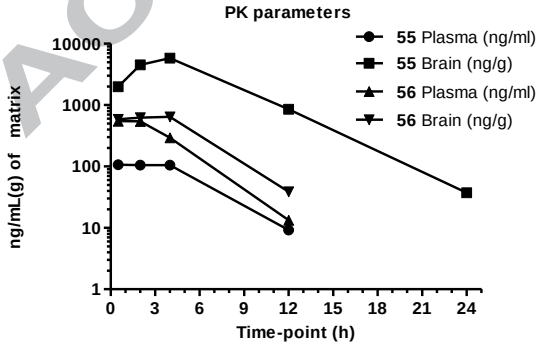


Figure 5: Brain and plasma levels of **55** and **56** after oral administration of 10 mg/kg.

In summary, we have identified a novel series of indole and 7-azaindole derivatives containing nitrile, piperidine and N-methyl piperidine substituents at the 3-position as inhibitors of Aβ aggregation (**45-57**) leading to improved metabolic stability in human and mice liver microsomes. The study described above indicated it was required to abandon the dimeric compound design of

libraries 2 and 3 to improve permeability and to avoid compounds being P-gp substrates. The introduction of the N-methyl piperidine moiety, together with the reduction of the hydrogen bond donors to two in library 4 was essential for good brain exposure in Swiss male mice after oral administration of compounds **55** and **56**. In contrast to compounds containing the 3-aminopyrazole[24] or 2,6-diaminopyridine moieties[25], it was possible to eliminate all nitrogen atoms acting as hydrogen bond acceptors for compounds **52**, **53**, **55-57** of library 4. Taken together, these results showed **56** to be the best compound in terms of inhibition of Aβ aggregation properties and brain uptake after oral administration.

Supplementary data

Supplementary (experimental procedures for the preparation of compounds **5-8**, **10-25, 27-30**, **32-57**, *in vitro* fluorescence assay) data associated with this article can be found, in the online version.

References and notes

1. Smith; M. A. *Int. Rev. Neurobiol.* **1998**, *42***,** 1
2. Selkoe, D. J. *J. Neuropathol. Exp. Neurol.* **1994,** *53***,** 438
3. Clark, C. M.; Karlawish, J. H. T. *Ann. Intern. Med.* **2003,** *138***,** 400
4. Rogers, S. L.; Doody, R. S.; Mohs, R. C.; Friedhoff, L. T. *Arch. Intern. Med.* **1998,** *158***,** 1021
5. Sabbagh, M.; Cummings, J. *BMC. Neurol.* **2011**, *11***,** 21
6. Vassar, R. *Adv. Drug Deliv. Rev* **2002,** *54***,** 1589
7. Vassar, R., Kovacs, D. M.; Yan, R.; Wong, P. C. *J. Neurosci.* **2009**, *29***,** 12787
8. Lim, G. P.; Yang, F.; Chu, T.; Chen, P.; Beech, W.; Teter, B.; Tran, T.; Ubeda, O.; Ashe, K. H.; Frautschy, S. A.; Cole, G. M. *J. Neurosci.* **2000**, *20*, 5709
9. Lorenzo, A.; Yankner, B. A. *Proc. Natl. Acad. Sci. U. S. A* **1994**, *91***,** 12243
10. Merlini, G.; Ascari, E.; Amboldi, N.; Bellotti, V.; Arbustini, E.; Perfetti, V.; Ferrari, M.; Zorzoli, I.; Marinone, M. G.; Garini, P. *Proc. Natl. Acad. Sci. U. S. A* **1995**, *92***,** 2959
11. Tomiyama, T.; Shoji, A.; Kataoka, K.; Suwa, Y.; Asano, S.; Kaneko, H.; Endo, N. *J. Biol. Chem* **1996**, *271***,** 6839
12. Wood, S. J.; Wetzel, R.; Martin, J. D.; Hurle, M. R. *Biochemistry* **1995**, *34***,** 724
13. Wood, S. J.; MacKenzie, L.; Maleeff, B.; Hurle, M. R.; Wetzel, R. *J. Biol. Chem*. **1996**, *271*, 4086
14. Ballatore, C.; Brunden, K. R.; Piscitelli, F.; James, M. J.; Crowe, A.; Yao, Y.; Hyde, E.; Trojanowski, J. Q.; Lee, V. M.-Y.; Smith, A. B., III *J. Med. Chem.* **2010**, *53***,** 3739
15. Ballatore, C.; Crowe, A.; Piscitelli, F.; James, M.; Lou, K.; Rossidivito, G.; Yao, Y.; Trojanowski, J. Q.; Lee, V. M.-Y.; Brunden, K. R.; Smith, A. B., III *Bioorg. Med. Chem*. **2012**, *20***,** 4451
16. Bulic, B.; Pickhardt, M.; Mandelkow, E. *J. Med. Chem*. **2013**, 56, 4135
17. Bulic, B.; Pickhardt, M.; Mandelkow, E. M.; Mandelkow, E. *Neuropharmacology* **2010**, *59***,** 276
18. Hensley, K.; Carney, J. M.; Mattson, M. P.; Aksenova, M.; Harris, M.; Wu, J. F.; Floyd, R. A.; Butterfield, D. A. *Proc. Natl. Acad. Sci. U. S. A* **1994**, *91***,** 3270
19. Cherny, R. A.; Atwood, C. S.; Xilinas, M. E.; Gray, D. N.; Jones, W. D.; McLean, C. A.; Barnham, K. J.; Volitakis, I.; Fraser, F. W.; Kim, Y.-S.; Huang, X.; Goldstein, L. E.; Moir, R. D.; Lim, J. T.; Beyreuther, K.; Zheng, H.; Tanzi, R. E.; Masters, C. L.; Bush, A. I. *Neuron* **2001**, *30***,** 665
20. Flood, D. G.; Reaume, A. G.; Dorfman, K. S.; Lin, Y. G.; Lang, D. M,; Trusko, S. P.; Savage, M. J.; Annaert, W. G.; De Strooper, B.; Siman, R.; Scott, R. W. *Neurobiol Aging* **2002**, *23***,** 335
21. Duff, K.; Suleman, F. *Brief. Funct. Genomic. Proteomic* **2004,** 3, 47
22. Rzepecki, P.; Nagel-Steger, L.; Feuerstein, S.; Linne, U.; Molt, O.; Zadmard, R.; Aschermann, K.; Wehner, M.; Schrader, T.; Riesner, D. *J. Biol. Chem*. **2004**, *279***,** 47497
23. Rzepecki, P.; Geib, N.; Peifer, M.; Biesemeier, F.; Schrader T. *J. Org. Chem*. **2007**, *72***,** 3614
24. Kroth, H.; Ansaloni, A.; Varisco, Y.; Jan, A.; Sreenivasachary, N.; Rezaei-Ghaleh, N.; Giriens, V.; Lohmann, S.; Lopez-Deber, M. P.; Adolfsson, O.; Pihlgren, M.; Paganetti, P.; Froestl, W.; Nagel-Steger, L.; Willbold, D.; Schrader, T.; Zweckstetter, M.; Pfeifer, A.; Lashuel, H. A.; Muhs, A. *J. Biol. Chem.* **2012**, *287*, 34786
25. Kroth, H.; Sreenivasachary, N.; Hamel, A.; Benderitter, P.; Varisco, Y.; Giriens V.; Paganetti, P.; Froestl, W.; Pfeifer, A.; Muhs, A. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3330
26. Bolder, S. G.; Sagis, L. M. C.; Venema, P.; van der Linden, E. *Langmuir* **2007**, *23***,** 4144
27. Levine, H., III *Protein Sci.* **1993**, *2*, 404
28. Jan, A.; Hartley, D. M.; Lashuel, H. A. *Nat. Protoc*. **2010**, *5*, 1186
29. Fors, B. P.; Krattiger, P.; Strieter, E.; Buchwald, S. L. *Org. Lett.* **2008**, *10*, 3505
30. Fors, B. P.; Watson, D. A.; Biscoe, M. R.; Buchwald, S. L. *J. Am. Chem Soc*. **2008**, *130*, 13552
31. Fors, B. P.; Buchwald, S. L. *J. Am. Chem. Soc*. **2010**, *132*, 15914
32. Storz, T.; Bartberger, M. D.; Sukits, S.; Wilde, C.; Soukup, T. *Synthesis* **2008**, 201
33. Mushtaq, N.; Saify, Z. S.; Noor, F.; Takween, S.; Akhtar, S.; Arif, M.; Khan, K. M. *Pak. J. Pharm. Sci.* **2008**, *21*, 36

Graphical Abstract

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Discovery and characterization of novel indole and 7-azaindole derivatives as inhibitors of β-amyloid-42 aggregation for the treatment of Alzheimer's disease

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