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Structure–Activity Relationships of Organofluorine Inhibitors of β-Amyloid Self-Assembly

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A broad group of structurally diverse small organofluorine compounds were synthesized and evaluated as inhibitors of β-amyloid (Aβ) self-assembly. The main goal was to generate a diverse library of compounds with the same functional group and to observe general structural features that characterize inhibitors of Aβ oligomer and fibril formation, ultimately identifying structures for further focused inhibitor design. The common structural motifs in these compounds are CF_3-C-OH and CF_3-C-NH groups that were proposed to be binding units in our previous studies. A broad range of potential small-molecule inhibitors were synthesized by combining various carbocyclic and heteroaromatic rings with an array of substituents, generating a total of 106 molecules. The compounds were tested by standard methods such as thioflavin-T fluorescence spectroscopy for monitoring fibril formation, biotinyl $A\beta_{1-42}$ single-site streptavidin-based assays for observing oligomer formation, and atomic force microscopy for morphological studies. These assays revealed a number of structures that show significant inhibition against either Aβ fibril or oligomer formation. A detailed analysis of the structure–activity relationship of anti-fibril and -oligomer properties is provided. These data present further experimental evidence for the distinct nature of fibril versus oligomer formation and indicate that the interaction of the Aβ peptide with chiral small molecules is not stereospecific in nature.

Introduction

Protein deposits in the form of neurofibrillary tangles and amyloid plaques are the hallmarks of Alzheimer's disease (AD).[1,2] The major component of extracellular amyloid plaques is the β-amyloid (Aβ) peptide.[3] One of the suggested therapeutic strategies for treating AD is inhibition of the amyloid cascade,[4] and many inhibitors of Aβ self-assembly have been identified. These include small organic molecules, peptides, peptidomimetics, and proteins.[5] Recent studies have indicated that the soluble oligomeric aggregates of Aβ are more neurotoxic than the fibrillar end-products of the process.[6] Therefore, it has become imperative to distinguish between molecules that inhibit oligomerization, fibril formation, or both. Many small-molecule anti-amyloidogenic compounds have been categorized, and the underlying oligomer structures characterized through the use of conformation-specific antibodies.[7–9] Several accounts[4,5,10] on the development of inhibitors active against fibrillogenesis and oligomer assembly serve as an excellent source of information. However, one cannot overlook the fact that the literature is far from systematic regarding the chemical nature of inhibitors. Most original studies focus on a single compound, or a small group of compounds with no clear indication as to why the compounds were selected. Moreover, rational extended structure–activity relationship studies outside the pharmaceutical industry are quite rare.[11,12] Although the target (fibrils, oligomers, etc.) is usually specified, frequently there is little indication about the type of interaction that occurs between the inhibitor and the peptide.

Our chemistry-based approach is intended to fill this gap. While many approaches in the search for potential inhibitors were discovery based, our design of a core structure was based on published data. In an earlier study we described a new class of organofluorine molecules as Aβ fibrillogenesis inhibitors.[13] These compounds have been found to be active in the disassembly of preformed fibrils as well.[14] In a study including chiral isomer pairs of the same compounds, it was also observed that individual chirality does not appear to result in a significant difference in the action of these compounds.[15] However, while providing interesting information and effective anti-fibril compounds, these studies were limited in scope; only a few compounds with closely related structural features were included. In continuation of our work on anti-AD compounds, we designed and synthesized a broad range of organofluorine molecules with one common motif (CF_3-C-XH,

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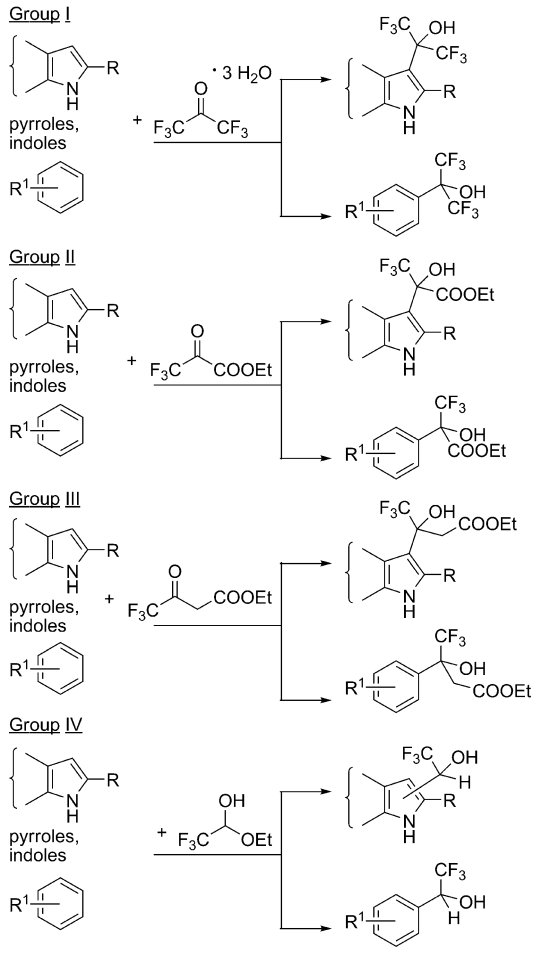
where X = O, N), yet considerable structural diversity. This functionality was found to be crucial for activity; removal of either the CF_3 or OH group resulted in completely inactive compounds.[13] The compounds were evaluated in fibril and oligomer inhibition and disassembly assays. Herein we describe a broad structure–activity relationship study of these organofluorine compounds as potential anti-AD agents.

Results

Various substituted/unsubstituted monocyclic/bicyclic aromatic/heteroaromatic molecules such as benzene, pyrrole, furan, and indole were derivatized by using commercially available trifluoromethyl hydroxyalkylating agents: ethyl trifluoropyruvate (TFP), ethyl trifluoroacetoacetate (TFAA), hexafluoroacetone (HFA), and trifluoroacetaldehyde ethylhemiacetal (TFAE). The basic synthetic procedures for the preparation of these compounds are summarized in Scheme 1.

A common feature to all syntheses in Scheme 1 is that every process occurs in one step, with commercially available hydroxyalkylating agents. In some cases, we used our own methods reported earlier;[16–18] for the remaining products, analogous published methods were applied.[19] A few selected chiral compounds were also synthesized by a cinchona-alkaloid-catalyzed hydroxyalkylation[18,20] to confirm the effect of inhibitor stereochemistry on anti-aggregation potency, especially because only the anti-fibril effect was described in our earlier report.[15]

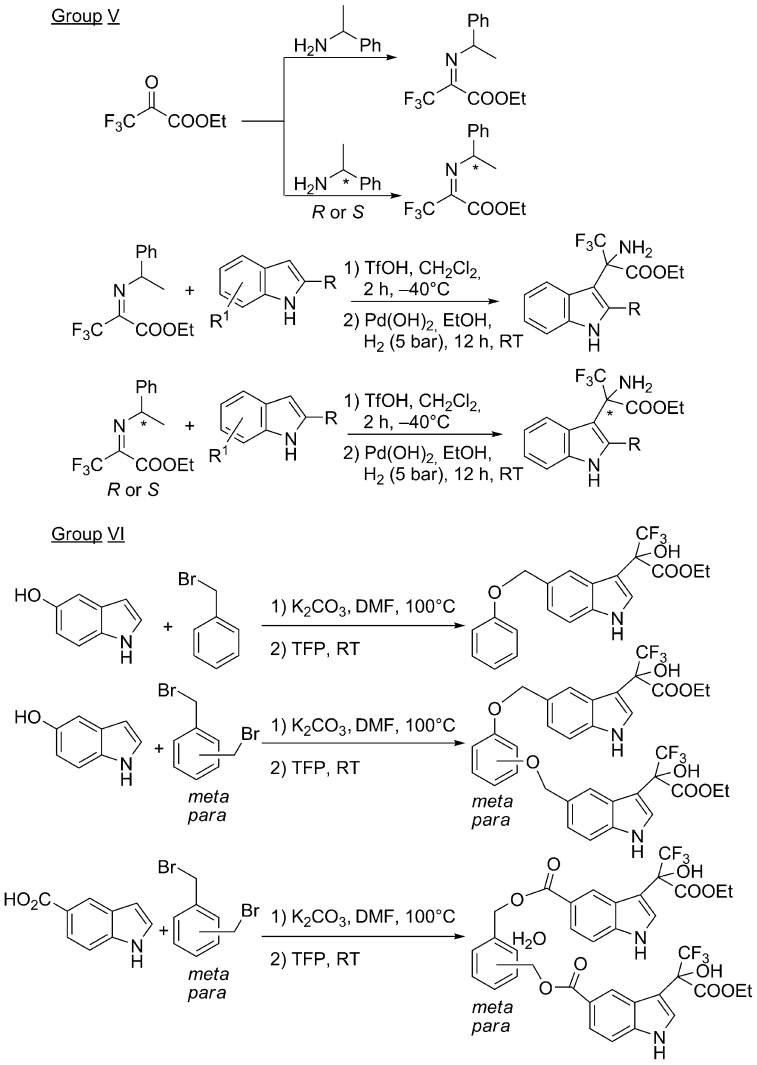


Scheme 1. One-step syntheses of a diverse series of aryl CF_3-C-OH group-containing compounds from commercially available reagents.

For the synthesis of trifluoromethyl amino acid esters and additional larger compounds, multistep methods were applied.[21,22] These procedures are summarized in Scheme 2. The syntheses provided the products in good to excellent yields, and in the case of the amino acid esters, the optical purities of the products were also high (up to 98% *ee*). Notably, several of these synthesized molecules are new compounds, whereas others were previously reported by our research group.[16–21] Therefore, other than the few compounds that appear in our previous publication on anti-fibrillogenesis compounds, none have been tested and described in Aβ aggregation inhibition studies until now.

Overall we completed the synthesis of 106 structurally related compounds. All these products possess a CF_3-C-OH or CF_3-C-NH unit as a common feature; otherwise the compounds are structurally diverse. It was our intention to observe the role that aromatic/heteroaromatic groups and their substituents may play in the course of fibril formation. In addition, the effect of double substitution was also tested, namely, whether the presence of two CF_3-C-OH units in one compound is beneficial or disadvantageous for the biological effect.

Following the syntheses, initial biochemical tests were carried out. Because both fibrillar aggregates and soluble oligomeric species of Aβ are neurotoxic, the inhibitory activity of the compounds was determined against both forms of self-assembly products. Our goal was to use $A\beta_{1-40}$, as it is the most abundant form of the peptide and readily forms fibrils. However, $A\beta_{1-42}$ was used for oligomers, because $A\beta_{1-40}$ forms oligomers poorly at the low concentration (10 nM) used to avoid fibril formation unless a stimulant is applied. The efficacy of the inhibitors against fibrillogenesis was evaluated by the commonly applied quantitative thioflavin-T (THT) fluorescence spectroscopy assay.[23–25] The calculated intensity values are based on maximum fluorescence intensities in the λ 480–490 nm emission region (λ_{ex} = 435 nm) after subtracting the background fluorescence of the starting solutions (0 h). The samples were incubated for four days, THT measurements were made at the plateau phase of fibril assembly, and the data obtained with the inhibitor-containing samples were compared with those of inhibitor-free controls. The assays were carried out using an Aβ/inhibitor ratio of 0.1 at an Aβ concentration of 100 μM; thus the original inhibitor concentration was at 1 mM (except compounds **89**–**93**, which were tested at a 1:1 molar ratio, thus at 100 μM, due to solubility problems). The data along with the compound structures are summarized in Figure 1. The anti-oligomer activities of the compounds were also determined using the quantitative biotinyl-$A\beta_{1-42}$ single-site streptavidin-based assay.[26,27] Samples were incubated for 30 min in the assays. These assays were carried out at an Aβ/inhibitor ratio of 0.0002, with Aβ at 0.01 μM. The efficacy of the compounds was determined at these given concentrations. The measured intensities of the inhibitor-containing samples (I_{sample}) were normalized to the con-



Scheme 2. Syntheses of aryl trifluoromethyl amino acid esters and larger compounds with CF_3-C-XH group (X = O, N) by multistep approaches.

trol sample ($I_{control}$) containing Aβ only. The percent values by which a compound decreased the expected signal (control) are listed in Figure 1 as percent inhibition.

$$\%\,\text{Inhibition} = 100 - \frac{I_{sample}}{I_{control}} \times 100 \quad (1)$$

In some cases compounds promoted self assembly, thus $I_{sample} > I_{control}$, and therefore negative percent inhibition values are listed. Compounds with significant activity in the screening assay were titrated, and the EC_{50} values were determined. The EC_{50} calculations were carried out as previously described.[13] Fluorescence intensity versus molar ratio functions were used to determine the relative potency of inhibitors using a simple equation, similar to the analysis of Michaelis–Menten kinetics or ligand binding to macromolecules [Eq. (2)]:[13]

$$I_{THT} = 100 - \frac{EC_{max}\,P}{EC_{50} + P} \quad (2)$$

in which I_{THT} is the fluorescence intensity of the inhibitor-containing sample expressed as a percentage of control, P is the inhibitor/Aβ molar ratio, EC_{50} is the median inhibitor constant, and EC_{max} is the maximum inhibition. A double-reciprocal plot of Equation (2) allows the determination of EC_{50}. Because inhibitor/Aβ molar ratios were applied in the formula, the EC_{50} values were obtained as a ratio as well. Multiplying the obtained ratio with the Aβ concentration of 100 μM provided the values in concentration units (μM).

The most active compounds and their EC_{50} data are listed in Table 1. The data show that Group I and II compounds are the most effective fibrillogenesis inhibitors, whereas inhibitors of oligomer assembly appear in several groups. A detailed analysis is provided in the Discussion section below to point out structural similarities among the active compounds. Atomic force microscopy (AFM) was also used to confirm the THT assay data. Several illustrative AFM images of a control sample and inhibitor samples are depicted in Figure 2.

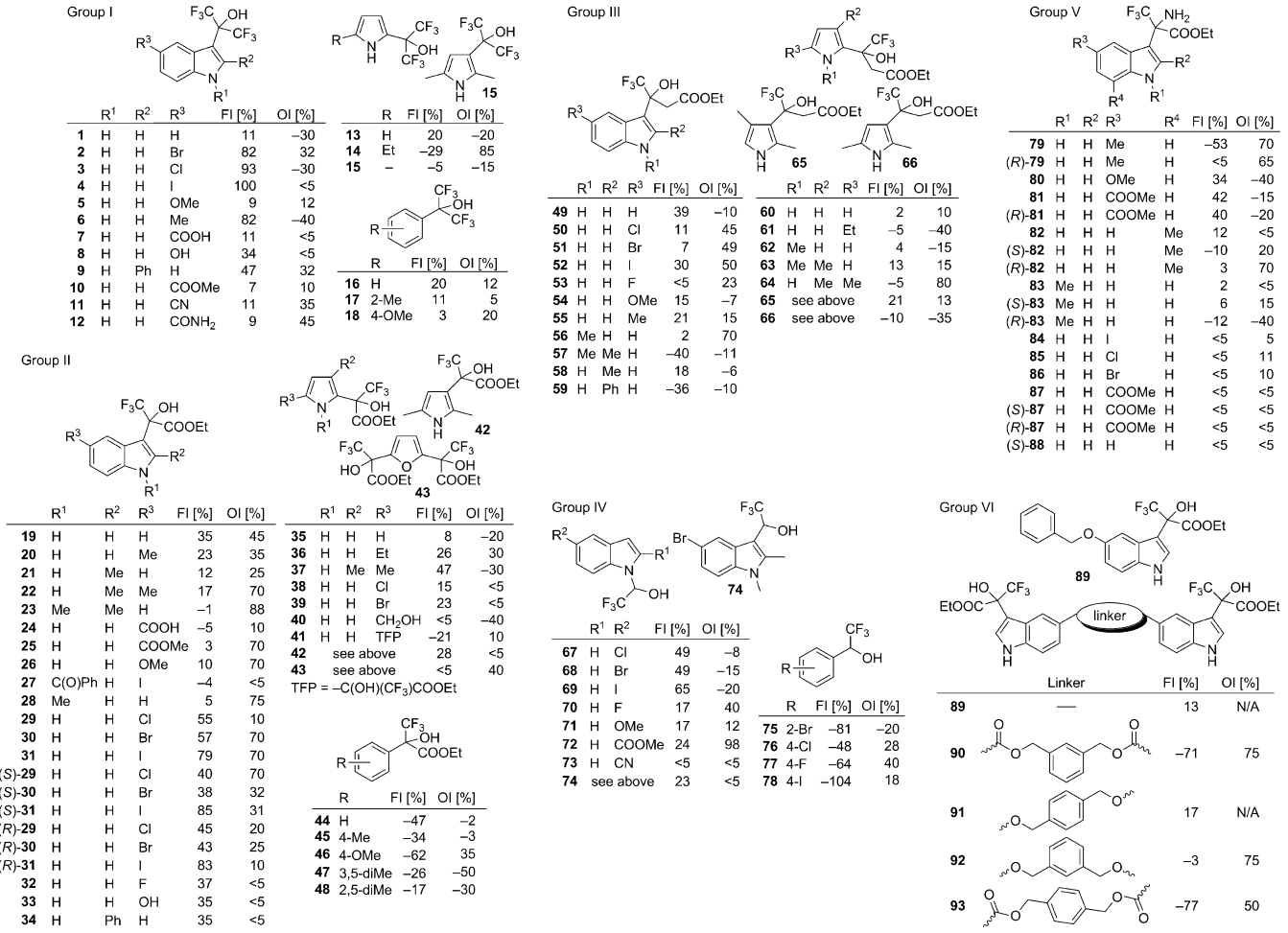
The data observed in the AFM images are in good agreement with the data from the THT fluorescence measurements (Figure 1). The control sample (Figure 2, control) shows the expected, well-developed network of mature fibrils. Images obtained in the presence of **1**, **5**, **44**, and **46**, individually, indicate extensive fibril formation and hence little inhibition. This is exactly what was observed in the quantitative assay; these compounds have practically no effect on fibril formation. Indeed the highest value, observed with **1** (11% inhibition), is still negligible. Compounds **44** and **46** were found to be promoters of fibril formation, resulting in visually denser fibrillar morphology.

Compounds **2**, **3**, and **4** possess similar structure; all of them are in Group I (Scheme 2, Figure 1). These compounds exhibited significant inhibition in the

Table 1. EC_{50} data for the most active compounds in Aβ fibrillogenesis and oligomer assembly assays.[a]

Compound	$EC_{50\ fibril}$ [μM]	$EC_{50\ oligomer}$ [μM]
2	380 ± 1.8	> 100
3	250 ± 4.7	N/A
4	190 ± 0.07	N/A
22	> 1000	53 ± 3.5
29	50[13]	> 100
30	20[13]	> 100
31	30[13]	> 100
43	> 1000	28 ± 2.8
64	N/A	15 ± 1.4
79	N/A	60 ± 10.6
90	N/A	19 ± 5.1
92	N/A	23 ± 4.9

[a] N/A: either no inhibition or promoter.



	R^1	R^2	R^3	FI [%]	OI [%]
1	H	H	H	11	–30
2	H	H	Br	82	32
3	H	H	Cl	93	–30
4	H	H	I	100	<5
5	H	H	OMe	9	12
6	H	H	Me	82	–40
7	H	H	COOH	11	<5
8	H	H	OH	34	<5
9	H	Ph	H	47	32
10	H	H	COOMe	7	10
11	H	H	CN	11	35
12	H	H	$CONH_2$	9	45

	R	FI [%]	OI [%]
13	H	20	–20
14	Et	–29	85
15	–	–5	–15

	R	FI [%]	OI [%]
16	H	20	12
17	2-Me	11	5
18	4-OMe	3	20

	R^1	R^2	R^3	FI [%]	OI [%]
19	H	H	H	35	45
20	H	H	Me	23	35
21	H	Me	H	12	25
22	H	Me	Me	17	70
23	Me	Me	H	–1	88
24	H	H	COOH	–5	10
25	H	H	COOMe	3	70
26	H	H	OMe	10	70
27	C(O)Ph	H	I	–4	<5
28	Me	H	H	5	75
29	H	H	Cl	55	10
30	H	H	Br	57	70
31	H	H	I	79	70
(*S*)-**29**	H	H	Cl	40	70
(*S*)-**30**	H	H	Br	38	32
(*S*)-**31**	H	H	I	85	31
(*R*)-**29**	H	H	Cl	45	20
(*R*)-**30**	H	H	Br	43	25
(*R*)-**31**	H	H	I	83	10
32	H	H	F	37	<5
33	H	H	OH	35	<5
34	H	Ph	H	35	<5

	R^1	R^2	R^3	FI [%]	OI [%]
35	H	H	H	8	–20
36	H	H	Et	26	30
37	H	Me	Me	47	–30
38	H	H	Cl	15	<5
39	H	H	Br	23	<5
40	H	H	CH_2OH	<5	–40
41	H	H	TFP	–21	10
42	see above			28	<5
43	see above			<5	40

	R	FI [%]	OI [%]
44	H	–47	–2
45	4-Me	–34	–3
46	4-OMe	–62	35
47	3,5-diMe	–26	–50
48	2,5-diMe	–17	–30

	R^1	R^2	R^3	FI [%]	OI [%]
49	H	H	H	39	–10
50	H	H	Cl	11	45
51	H	H	Br	7	49
52	H	H	I	30	50
53	H	H	F	<5	23
54	H	H	OMe	15	–7
55	H	H	Me	21	15
56	Me	H	H	2	70
57	Me	Me	H	–40	–11
58	H	Me	H	18	–6
59	H	Ph	H	–36	–10

	R^1	R^2	R^3	FI [%]	OI [%]
60	H	H	H	2	10
61	H	H	Et	–5	–40
62	Me	H	H	4	–15
63	Me	Me	H	13	15
64	H	Me	Me	–5	80
65	see above			21	13
66	see above			–10	–35

	R^1	R^2	FI [%]	OI [%]
67	H	Cl	49	–8
68	H	Br	49	–15
69	H	I	65	–20
70	H	F	17	40
71	H	OMe	17	12
72	H	COOMe	24	98
73	H	CN	<5	<5
74	see above		23	<5

	R	FI [%]	OI [%]
75	2-Br	–81	–20
76	4-Cl	–48	28
77	4-F	–64	40
78	4-I	–104	18

	R^1	R^2	R^3	R^4	FI [%]	OI [%]
79	H	H	Me	H	–53	70
(*R*)-**79**	H	H	Me	H	<5	65
80	H	H	OMe	H	34	–40
81	H	H	COOMe	H	42	–15
(*R*)-**81**	H	H	COOMe	H	40	–20
82	H	H	H	Me	12	<5
(*S*)-**82**	H	H	H	Me	–10	20
(*R*)-**82**	H	H	H	Me	3	70
83	Me	H	H	H	2	<5
(*S*)-**83**	Me	H	H	H	6	15
(*R*)-**83**	Me	H	H	H	–12	–40
84	H	H	I	H	<5	5
85	H	H	Cl	H	<5	11
86	H	H	Br	H	<5	10
87	H	H	COOMe	H	<5	<5
(*S*)-**87**	H	H	COOMe	H	<5	<5
(*R*)-**87**	H	H	COOMe	H	<5	<5
(*S*)-**88**	H	H	H	H	<5	<5

	Linker	FI [%]	OI [%]
89	—	13	N/A
90		–71	75
91		17	N/A
92		–3	75
93		–77	50

Figure 1. General structure of the compounds used in the current study and their activity in Aβ aggregation assays (FI: fibril inhibition; OI: oligomer inhibition).

THT assays in increasing order (**2**: 82%, **3**: 93%, **4**: 100%), and this trend can be followed in Figure 2 as well. Ever fewer fibrils appear in the images from **2** to **3**, and there are practically no fibrils in the presence of compound **4**. Other compounds (**8** and **9**) showed moderate inhibition (34–47%), and this is reflected in the AFM images. The dense fibrillar network characteristic of the control is less frequent with these compounds; however, a significant amount of fibrils is still present in the images. Visual analysis of images obtained with other inhibitors also indicate strong inhibition (compounds **37**, **42**, **67**, **69**, **80**, and **81**). It also reveals that in the presence of inhibitors, aggregates with various morphologies can form. In many cases the obtained fibrils are nearly identical to those observed in the control sample, although less dense in appearance. In some cases (Figure 2: **3**, **67**, and **69**) only a few identifiable fibrils are present. In other cases, however, the aggregates appear short (Figure 2: **42** and **80**) or emerge in the form of round-shaped deposits (Figure 2: **81**), indicating that strong polymorphism can occur from the presence of these inhibitors.

Discussion

Analysis of the above data indicates that several compounds in the synthesized compound library show strong activity against the formation of either fibrils or oligomers. This phenomenon appears to support earlier suggestions that not all stable oligomers are obligatory precursors to the fibrils and that the two processes can occur in parallel pathways. Therefore, a given compound may affect one process or the other, and not necessarily both.[7–9] The structure–activity relationships are discussed separately for fibril and oligomer inhibition.

Fibrillogenesis inhibitors

The most potent fibril inhibitors were compounds **2**–**4** and **6** from Group I, bearing the $(CF_3)_2$-C-OH motif, whereas several CF_3(COOEt)-C-OH-containing compounds (**31**, (*S*)-**31**, and (*R*)-**31**) showed the strongest and nearly identical activity. Other functional groups typically imparted weaker inhibitory potential, with inhibition values up to ~50%. These data are consistent with our previous hypothesis that the acidity of the inhibi-

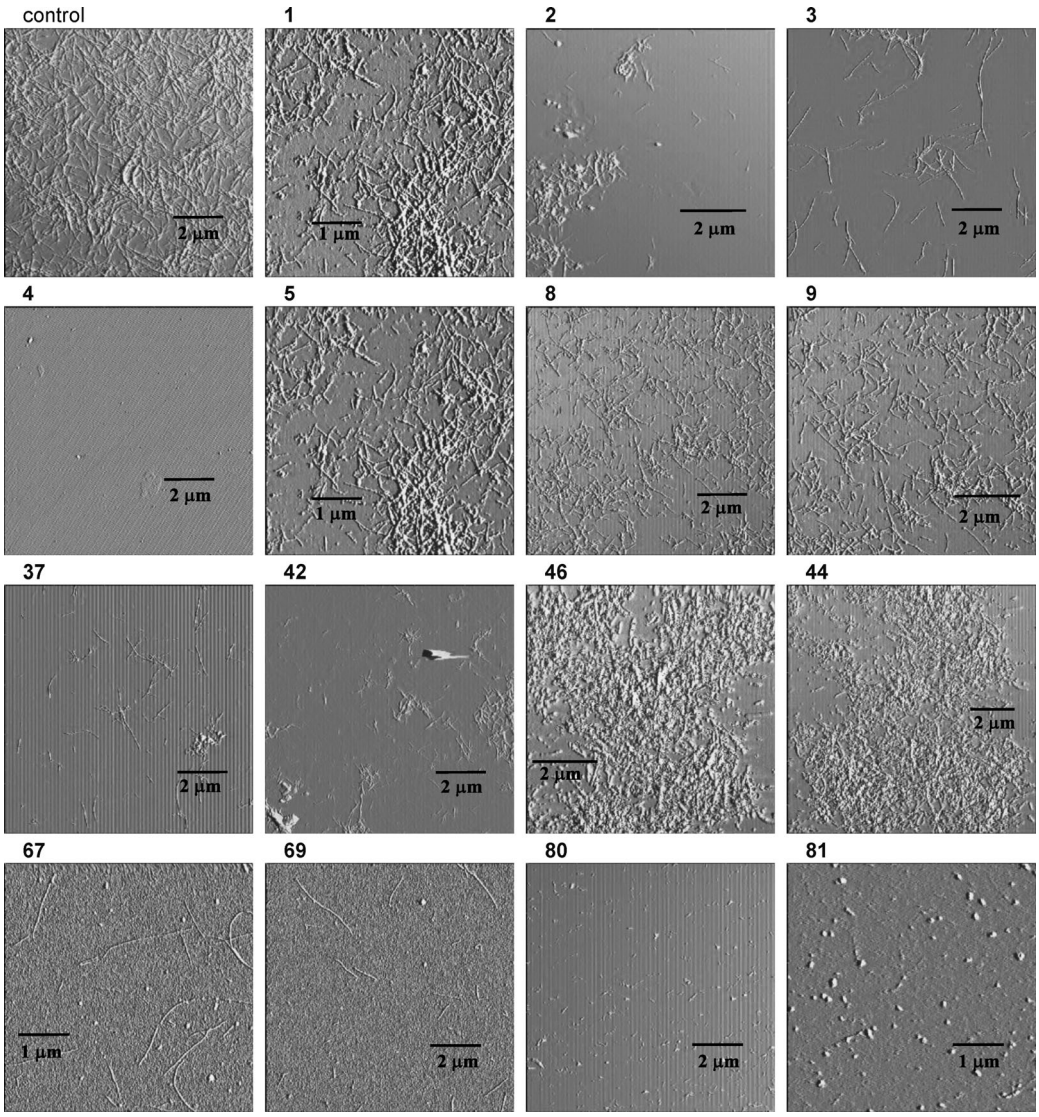


Figure 2. Atomic force microscopy images of $A\beta_{1-40}$ samples incubated without (control) and with the indicated test compounds (see Figure 3) for four days.

tors is a crucial factor in the mode of action of these compounds; the more acidic the OH group, the greater the potency. The order of acidic strength and, in parallel, inhibitor activity of the various motifs used is illustrated in Figure 3.

The role of the aromatic groups also appears important. In every group of compounds tested, the indolyl derivatives were found to be the most active followed by the pyrrole-based compounds. The weakest (or no) effect was consistently observed for the simple carbocyclic molecules (benzene derivatives). This observation highlights the significance of the heterocyclic aryl group, particularly indole over carbocyclic rings. Notably, whereas the carbocyclic derivatives of Group I (**16**–**18**) showed weak inhibitory effects, similar compounds from all other groups were found to be self-assembly promoters (**44**–**48** and **75**–**78**). In summary, indole-based inhibitors showed strong fibril-inhibition properties, while other aromatic units were modest inhibitors at best, and often acted as promoters of fibrillogenesis.

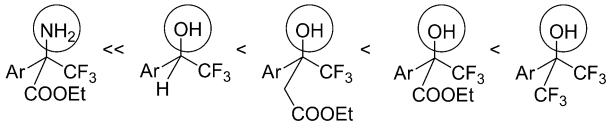


Figure 3. The rank order of acid strength of the major types of inhibitors used.

Based on this observation, our discussion on the role of individual substituents is focused on indole derivatives. Group I and II compounds are the most promising fibril inhibitors; the general activities of other compounds, while consistent, show a steady decrease as a function of the acidity of the CF_3-C-XH group (X = O, N; Figure 3). For Group II compounds, the 5-halogen-substituted (R^3 substituent) indoles were found to be the best inhibitors, in the order of F < Cl < Br < I. The same order was observed in the case of the newly synthesized Group I molecules. Compound **4** (R^3 = I) showed 100% fibril inhibition

under the experimental conditions, whereas the Br- and Cl-substituted inhibitors showed decreasing but still high efficacy (93 and 82%). Therefore, it appears that the presence of a larger halogen atom at the R^3 position of the indole ring increases the inhibitory activity toward fibril formation. However, substitution of the halogens with bulky electron-withdrawing groups such as -COOMe, -CN, and -$CONH_2$ at the R^3 position of indoles decreases the ability of these compounds to inhibit the formation of fibrils. Although substituents at the other positions show minor effects, they do not appear to significantly alter inhibitor activity.

Oligomerization inhibitors

Analysis of the molecular features that result in effective oligomer inhibitors leads to the observation that, in contrast to fibrillogenesis inhibition, the acidity of the CF_3-C-OH motif does not appear to be of primary importance. Interestingly, the typically good fibril inhibitors (Groups I and II) show poor performance in oligomer inhibition assays, providing further evidence in support of the earlier findings that fibrils and stable oligomers do not form via the same pathways.[7,8] In fact, in certain cases these compounds promote oligomer formation. Effective oligomer inhibitors were found in all groups except Group I ((CF_3)$_2$-C-OH derivatives). Interestingly, from the groups of typical fibril inhibitors (I and II) only one compound showed significant oligomer inhibition (**22**, $EC_{50} = 53$ μM), and this compound is a weak fibril inhibitor. The most active inhibitors of oligomer assembly are those with multiple active CF_3-C-OH substituents (**43**, **90**, and **92**), showing better EC_{50} values (19–25 μM). Similar to the fibrillogenesis inhibitors, compounds with carbocyclic (benzene) rings are inactive in the inhibition of oligomer assembly. All effective inhibitors possess heterocyclic rings. Based on the molecular structures of compounds listed in Table 1 for oligomer inhibitors, a single, well-defined relationship cannot be made. However, the double CF_3-C-OH units, and in certain cases the larger size or more aromatic rings, suggest that for oligomer inhibition the presence of aromatic groups and the possibility of π–π interactions[28–30] is more likely to be a decisive feature than the presence of acidic groups. It is also supported by data obtained with the carbocyclic compounds. These compounds (**44**–**48** and **75**–**78**) mostly promoted fibril formation, in contrast to their mild-to-moderate (76–28% and 77–40%, respectively) inhibition of oligomer assembly, highlighting the importance of π–π stacking.[28–30]

The effect of chirality on the inhibition of Aβ self-assembly has also attracted considerable attention.[15,28,31,32] Earlier results obtained with peptide-based[28] versus small-molecule inhibitors[15,31] appear to be controversial. While the individual (amino acid) chirality is of key importance for peptide inhibitors,[28,32] it did not appear to be so for small-molecule inhibitors. In a recent work it was stated that chirality is an important feature for methoxytacripyrines as inhibitors.[33] Analysis of the published data, however, led us to the conclusion that the activity differences between the enantiomers or racemic mixtures, while considerably high for human acetylcholinesterase (*h*AChE) inhibition, are rather insignificant (< 10%) for inhibition of Aβ assembly, given the experimental error of the fibril growth and analytical processes. Chiral compounds in our current set of molecules are included in Groups II and V. The results obtained with these compounds support our earlier conclusions.[15] The differences between the inhibitory effect of enantiomers and racemic mixtures mostly fall within a 0–20% range (29–5%, 30–7%, 31–2%, 82–13%, and 83–18%), although in a few cases it is greater than that. Notably, similar observations were made in the inhibition of both fibrillogenesis and oligomer assembly. Although more data using structurally diverse compounds are clearly needed to provide a definite answer to this problem, the results presented herein suggest that the interaction of chiral small molecules with Aβ is most likely not stereo- or enantiospecific.

Conclusions

In summary, the synthesis and activity evaluation of a set of 106 structurally diverse compounds with the same motif (CF_3-C-X; X = OH, NH_2) resulted in valuable information for the further design of Aβ self-assembly inhibitors. An earlier observation regarding the importance of the acidity of the OH group in fibrillogenesis inhibition was confirmed, and new lead compounds **2**–**4** were identified. It was also observed that acidity is a relatively unimportant characteristic of the compounds in the inhibition of oligomer assembly. Instead, active oligomer inhibitors feature dual binding groups and more electron-rich aromatic units, emphasizing that ability to participate in π–π interactions is also a dominant aspect of these compounds.

Experimental Section

Chemistry

General information: The cinchona alkaloids were purchased from Fluka and used without further purification. Indole derivatives and ethyl 3,3,3-trifluoropyruvate, hexafluoroacetone trihydrate, trifluoroacetaldehyde ethylhemiacetal, substituted anilines, and benzaldehydes were obtained from Aldrich. $CDCl_3$ used as a solvent (99.8%) for NMR studies was from Cambridge Isotope Laboratories. The ^{19}F NMR reference compound $CFCl_3$ was purchased from Aldrich. Other solvents used in synthesis, with minimum purity of 99.5%, were purchased from Fisher. K-10 montmorillonite, a solid acid used as catalyst, was obtained from Fluka. Mass spectrometric identification of the products was carried out with an Agilent 6850 gas chromatograph – 5973 mass spectrometer system (70 eV electron impact ionization) using a 30-m-long DB-5 column (J&W Scientific). ^1H, ^{13}C, and ^{19}F NMR spectra were obtained on a 300 MHz superconducting Varian Gemini 300 NMR spectrometer, in $CDCl_3$ solvent with tetramethylsilane and CCl_3F as internal standards. The temperature was kept at $25 \pm 1\,^{\circ}C$ by a Varian temperature control unit. Determination of enantiomeric excess was carried out by chiral HPLC analysis using a Jasco PU-2080 HPLC coupled with a PU-2075 UV/Vis detector. The samples were analyzed in hexane/*i*PrOH (95:5) mobile phase using a Chiralcel OJ-H (Daicel) analytical column at λ 260 nm.

Synthesis of inhibitor candidates: Compounds used in this study were synthesized using published methods[16–22] or as described below. In each case, compounds were purified by flash chromatog-

raphy or preparative thin-layer chromatography (TLC). The identification and purity determination were carried out by GC–MS and NMR spectroscopy (^{1}H, ^{13}C, and ^{19}F when applicable). The known compounds showed NMR and MS characteristics identical to published data (see Supporting Information).

General procedure for the synthesis of 1,1,1,3,3,3-hexafluoro-2-(indol-3-yl)propan-2-ols 1–12: A microwave reaction vial containing molecular sieves (4 Å, 200 mg) was charged with indole (1 mmol) and HFA·3 H_2O (1.5 mmol, 205 μL). The contents of the vial were irradiated in a CEM Discover microwave reactor for 10 min at 100 °C. Reaction progress was monitored by GC–MS. After reaction completion, CH_2Cl_2 was added to the mixture. The content of the vial was filtered into a round-bottom flask and concentrated in vacuo. The products were isolated as crystals or oils and purified by flash chromatography if the purity by GC–MS was > 98 %.

General procedure for the synthesis of 1,1,1,3,3,3-hexafluoro-2-(1*H*-pyrrol-2-yl)propan-2-ols 13–15: A microwave reaction vial containing molecular sieves (4 Å, 200 mg) was charged with pyrrole (1 mmol) and HFA·3 H_2O (1.5 mmol, 205 μL). The content of the vial was irradiated in a CEM Discover microwave reactor for 10 min at 80 °C. Reaction progress was monitored by GC–MS. After reaction completion, CH_2Cl_2 was added to the mixture. The content of the vial was filtered into a round-bottom flask and concentrated in vacuo. The products were isolated as crystals or oils and purified by flash chromatography if the purity by GC–MS was > 98 %.

General procedure for the synthesis of 1,1,1,3,3,3-hexafluoro-2-phenylpropan-2-ols 16–18: Arene (1 mmol) and TFP (1.5 mmol) were added to a dry pressure tube containing CH_2Cl_2 (3 mL) under N_2 atmosphere. Trifluoromethanesulfonic acid (50 mol %) was added to the reaction mixture dropwise. The reaction mixture was stirred for 16 h, after which the contents were poured into 5 mL H_2O and extracted with CH_2Cl_2. After evaporation of solvents, the residue was purified by flash chromatography to give the final product.

General procedure for the synthesis of 3,3,3-trifluoro-2-hydroxy-2-(indol-3-yl)propionic acid ethyl esters 19–34: The compounds were synthesized based on a published method.[16] Indole (0.75 mmol), ethyl 3,3,3-trifluoropyruvate (TFP, 1.125 mmol), and K-10 montmorillonite (500 mg) were mixed in toluene (3 mL) in a Teflon screw-cap pressure tube. The reaction mixture was heated and stirred at 60 °C, and reaction progress was monitored by TLC. After satisfactory conversion, the product mixture was separated from catalyst by filtration. The solvent and excess TFP were removed under vacuum. The products were isolated as crystals or oils and purified by flash chromatography. Pyrrole derivatives **35–43** were prepared by the same method.

General procedure for the synthesis of ethyl 3,3,3-trifluoro-2-hydroxy-2-(1*H*-pyrrol-2-yl)propanoates 35–43: Pyrrole (0.5 mmol) and ethyl 3,3,3-trifluoropyruvate (TFP, 0.51 mmol) were mixed in a round-bottom flask. The reaction mixture was stirred at room temperature, and reaction progress was monitored by GC. After satisfactory conversion, the product mixture was extracted into CH_2Cl_2. The solvent and excess TFP were removed under vacuum. The product was then purified by flash chromatography.

General procedure for the synthesis of enantiomeric 3,3,3-trifluoro-2-hydroxy-2-(indol-3-yl)propionic acid ethyl esters 29–31 (*S* and *R*): The enantiomers were prepared by an earlier cinchona-alkaloid-catalyzed organocatalytic method. Indole (0.5 mmol) and cinchonidine (for *S* products) or cinchonine (for *R* products) (0.0375 mmol) were placed into a glass reaction vessel and Et_2O (3 mL) was added. The mixture was stirred at −8 °C (salt–ice cooling bath) for 30 min. Ethyl 3,3,3-trifluoropyruvate (0.75 mmol) was then added, and the mixture was stirred at −8 °C (salt–ice cooling bath) for an additional 3 h, and the progress was monitored by TLC. After reaction completion, the solvent and excess TFP were removed by evaporation. The mixture was then dissolved in Et_2O, and the cinchona catalyst was removed by a treatment with K-10 montmorillonite (500 mg; a solid acid). After treatment, the alkaloid–K-10 complex was removed by filtration, and the solvent was evaporated. A colorless solid was obtained in 98 % yield. The enantiomeric excess of the product was determined by HPLC (see below). The product purity was > 98 % (86–93 % *ee*).

General procedure for the synthesis of ethyl 3,3,3-trifluoro-2-hydroxy-2-phenylpropanoates 44–48: Arene (1 mmol) and HFA·3 H_2O (1.5 mmol) were added to a pressure tube containing CH_2Cl_2 (3 mL) under N_2 atmosphere. Trifluoromethanesulfonic acid (2 equiv) was added to the reaction mixture dropwise. The reaction mixture was stirred for 16 h, after which the contents were poured into 5 mL H_2O and extracted into CH_2Cl_2. After the evaporation of solvents, the residue was purified by flash chromatography to give the final product.

General procedure for the synthesis of ethyl 4,4,4-trifluoro-3-hydroxy-3-(1*H*-indol-3-yl)butanoates 49–59: A solution of indole (0.5 mmol), ethyl 4,4,4-trifluoro-3-oxobutanoate (TFAA, 0.5 mmol) and K-10 montmorillonite was mixed in toluene (3 mL) in a Teflon screw-cap pressure tube. The reaction mixture was heated and stirred at 60 °C, and reaction progress was monitored by TLC. After satisfactory conversion, the product mixture was separated from catalyst by filtration. The product was purified by flash chromatography and isolated as crystals or oils.

General procedure for the synthesis of ethyl 4,4,4-trifluoro-3-hydroxy-3-(1*H*-pyrrol-2-yl)butanoates 60–66: A solution of pyrrole (0.5 mmol), TFAA (0.5 mmol) and K-10 montmorillonite was mixed in toluene (3 mL) in a Teflon screw-cap pressure tube. The reaction mixture was heated and stirred at 60 °C, and reaction progress was monitored by TLC. After satisfactory conversion, the product mixture was separated from catalyst by filtration. The product was purified by flash chromatography and isolated as crystals or oils.

General procedure for the synthesis of 1-(1*H*-indol-1-yl)-2,2,2-trifluoroethanols 67–73: A solution of indole (0.5 mmol), TFAE (2 mmol), and Et_3N (0.05 mmol) in DMF (0.25 mL) was irradiated in a CEM Discover microwave reactor for 20 min at 150 °C. The reaction mixture was then quenched with 10 mL H_2O, and the product was extracted with EtOAc (3 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, evaporated, and the product was isolated and purified by preparative TLC or column chromatography.

Synthesis of 1-(5-bromo-1*H*-indol-3-yl)-2,2,2-trifluoroethanol (74): A mixture of 5-bromoindole (0.5 mmol) and trifluoroacetaldehyde methylhemiacetal (1 mmol) was irradiated in a CEM Discover microwave reactor for 10 min at 100 °C. The reaction mixture was then directly subjected to preparative TLC for purification and product isolation. Isolated yield: 72 %; colorless solid, mp: 113–115 °C; ^{1}H NMR (300.128 MHz, $CDCl_3$): δ = 7.76 (m, 1 H), 7.33–7.37 (m, 3 H), 6.57 (d, J = 3.3 Hz, 1 H), 6.09 (p, J = 4.8 Hz, 1 H), 3.88 ppm (d, J = 4.8 Hz, 1 H); ^{13}C NMR (75.474 MHz, $CDCl_3$): δ = 130.68, 125.69, 125.58, 123.82, 120.30, 116.96, 114.13, 111.16, 104.23, 76.65 ppm (q, J = 36 Hz); ^{19}F NMR (300.128 MHz, $CDCl_3$): δ = −77.63 (d, J = 4.8 Hz); MS $C_{10}H_7BrF_3NO$ (294) *m/z* (%): 293 ([*M*]$^+$, 100), 295 ([*M*]$^+$, 98), 214 (30), 175 (25).

General procedure for the synthesis of 2,2,2-trifluoro-1-phenylethanols 75–78: Arene (1 mmol) and trifuoroacetaldehyde hemiacetal (TFAE; 1.5 mmol) were added to a pressure tube containing CH_2Cl_2 (3 mL) under N_2 atmosphere. Trifluoromethanesulfonic acid (2 equiv) was added to the reaction mixture dropwise. The reaction mixture was stirred for 16 h, after which the contents were poured into 5 mL H_2O and extracted with CH_2Cl_2. After evaporation of solvents, the residue was purified by flash chromatography to give the final product.

General procedure for the synthesis of ethyl 2-amino-3,3,3-trifluoro-2-(1*H*-indol-3-yl)propanoates 79–88: *Step 1*. General procedure for the preparation of ethyl 3,3,3-trifluoro-2-(1-phenylethylimino)propanoate: Montmorillonite K-10 (4 g) and toluene (20 mL) were placed into a round-bottom flask equipped with a stir bar, a reflux condenser, and a dry tube. TFP (6.24 mL, 0.047 mol) and α-methylbenzylamine (5 mL, 0.039 mol; racemic, *R*, or *S*) was dissolved in toluene (5 mL), and this solution was added to the above mixture. The reaction mixture was stirred at 100 °C for 4 h, and reaction progress was monitored by TLC. After the reaction completion, the resulting mixture was filtered through a sintered glass funnel and washed with CH_2Cl_2. The solvent and excess TFP were removed in vacuo to obtain a brown oil, which was later subjected to column chromatography (hexane/EtOAc 90:10) to obtain a colorless liquid in 92% isolated yield.

General procedure for synthesis of racemic 3,3,3-trifluoro-2-(1*H*-indol-3-yl)-2-(1-phenylethylamino)propanoates: *Step 2*. Racemic trifluoroimine, synthesized in Step 1 (300 mg, 1.09 mmol) and indole (0.98 mmol) were dissolved in 2 mL CH_2Cl_2. The reaction vessel was placed into an ice bath, and the mixture was stirred at 0 °C for 5 min. Trifluoromethanesulfonic acid (0.40 mmol, 20% solution in CH_2Cl_2) was added dropwise to the reaction mixture over a period of 15 min. After complete addition, the reaction mixture was stirred at 0 °C for another 2 h, and reaction progress was followed by TLC. After reaction completion, H_2O (5 mL) was added, and the reaction mixture was stirred at room temperature for 5 min to quench the acid. The resulting mixture was extracted with CH_2Cl_2, and the organic layer was washed with H_2O three times. The organic layers were combined, dried over Na_2SO_4, and filtered. The solvent was removed by evaporation, and the resulting crude mixture was purified by column chromatography.

General procedure for synthesis of chiral 3,3,3-trifluoro-2-(1*H*-indol-3-yl)-2-(1-phenylethylamino)propanoates (*R* or *S*): Trifluoromethylated imine (300 mg, 1.09 mmol) and indole (0.98 mmol) were dissolved in 2 mL CH_2Cl_2. The reaction vessel was placed into a cooling bath (EtOH/dry ice mixture) and the mixture was stirred at −40 °C for 15 min. Trifluoromethanesulfonic acid (0.40 mmol, 20% solution in CH_2Cl_2) was added dropwise to the reaction mixture over a period of 15 min. After complete addition, the reaction mixture was stirred at −40 °C for another 2 h, and reaction progress was followed by TLC. After the reaction completion, H_2O (5 mL) was added, and the reaction mixture was stirred at room temperature for 5 min to quench the acid. The resulting mixture was extracted into CH_2Cl_2, and the organic layer was washed three times with H_2O. The organic layers were combined, dried over Na_2SO_4, and filtered. The solvent was removed by evaporation, and the resulting crude mixture was purified by column chromatography.

General procedure for synthesis of ethyl 2-amino-3,3,3-trifluoro-2-(1*H*-indol-3-yl)propanoate (hydrogenolysis): *Step 3*. 3,3,3-Trifluoro-2-(1*H*-indol-3-yl)-2-(1-phenylethylamino)propanoate (150 mg, 0.38 mmol) was dissolved in EtOH (2 mL) along with $Pd(OH)_2$ (Pearlman's catalyst; 75 mg). The mixture was stirred under H_2 (5 bar) at room temperature for 12 h. After the reaction completion, the catalyst was separated by filtration. The resulting filtrate was concentrated in vacuo and purified by column chromatography.

Synthesis of 3,3,3-trifluoro-2-hydroxy-2-(5-benzyloxyindol-3-yl)-propionic acid ethyl ester (89): A solution of 5-hydroxyindole (66.5 mg, 0.5 mmol), benzyl bromide (65 μL, 0.55 mmol), and K_2CO_3 (276 mg, 2 mmol) in DMF (0.5 mL) was stirred for 24 h. TFP (192 μL, 1.4 mmol) was then added, and the mixture was stirred continuously for another 24 h. The reaction was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3×5 mL). Combined organic extracts were dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo. The residue was then subjected to column chromatography to afford pure product (170 mg, 87% yield).

General procedure for the synthesis of 1,3- and 1,4-phenylenebis(methylene)bis[3-(3-ethoxy-1,1,1-trifluoro-2-hydroxy-3-oxopropan-2-yl)-1*H*-indole-5-carboxylates] 90 and 93: A solution of 5-indole carboxylic acid (80.5 mg, 0.5 mmol), α,α′-dibromoxylene (*meta* or *para*, 66 mg, 0.25 mmol), and K_2CO_3 (276 mg, 2 mmol) in 0.5 mL DMF was stirred for 24 h. TFP (192 μL, 1.4 mmol) was then added and stirred for 24 h. The reaction was quenched with H_2O (10 mL), and the mixture was extracted with CH_2Cl_2 (3×5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography to afford pure **90** (130 mg, 68% yield) or **93** (135 mg, 71% yield).

General procedure for the synthesis of diethyl 2,2′-(5,5′-(1,3- and 1,4-phenylenebis(methylene))bis(oxy)bis(1H-indole-5,3-diyl))bis (3,3,3-trifluoro-2-hydroxypropanoates 91 and 92: A solution of 5-hydroxyindole (66.5 mg, 0.5 mmol), α,α′-dibromoxylene (*meta* or *para*, 66 mg, 0.25 mmol) and K_2CO_3 (276 mg, 2 mmol) in 0.5 mL CH_3CN was stirred for 24 h. TFP (192 μL, 1.4 mmol) was then added, and stirring was continued for another 24 h. The reaction was diluted with 10 mL H_2O and extracted with CH_2Cl_2 (3× 5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo. The crude products were subjected to column chromatography to yield pure **91** (143 mg, 81% yield) or **92** (138 mg, 78% yield).

Biochemical assays

General information: *Fibril assays*: NaH_2PO_4, Na_2HPO_4, NaN_3, NaOH, NaCl, glycine, DMSO, and thioflavin-T were obtained from Sigma–Aldrich. Lyophilized $A\beta_{1-40}$ peptide (purity >95%) was purchased from AnaSpec (Fremont, CA, USA). Mica sheets for AFM measurements were obtained from Alfa Aesar.

Oligomer assays: 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), DMSO, fluorescamine, ultrapure Tween 20, tetramethylbenzidine (free base), *N*,*N*-dimethylacetamide, tetrabutylammonium borohydride, and H_2O_2 (30% *w*/*w*) were obtained from Sigma–Aldrich. *N*-α-Biotinyl-$A\beta_{1-42}$ (bio-Aβ42) was purchased from AnaSpec. Fatty-acid-free fraction V bovine serum albumin was obtained from Boehringer–Mannheim. Streptavidin–HRP (SA–HRP) was obtained from Rockland. NeutrAvidin (NA) was obtained from Pierce. High-binding 9018 ELISA plates were purchased from Costar.

Thioflavin-T fluorescence assay for the determination of inhibitor activity in Aβ fibrillogenesis: Assays were carried out using a standard procedure.[23–25] The synthetic lyophilized $A\beta_{1-40}$ peptide was dissolved in 100 mM NaOH to a concentration of 40 mg mL^{-1} and diluted in 10 mM HEPES, 100 mM NaCl, 0.02% NaN_3 (pH 7.4)

buffer to a final peptide concentration of 100 μM. The use of NaOH as an initial solvent ensures that the isoelectric point of Aβ is bypassed, and the peptide remains in monomeric form.[34,35] Inhibitors were dissolved in DMSO to achieve a concentration of 0.15 M and added to the Aβ samples in HEPES buffer (inhibitor/Aβ = 10) to attain a final concentration of 1 mM. After vigorous vortexing for 30 s, the solutions were incubated at 37 °C with gentle shaking (77 rpm) for seven days, and the increase in fibril amount in each sample was followed by thioflavin-T fluorescence using the peptide without inhibitor as the control. The fluorescence measurements were carried out using a Hitachi F-2500 fluorescence spectrophotometer. The incubated peptide solutions were briefly vortexed before each measurement, and then 3.5 μL aliquots of the suspended fibrils were withdrawn and added into 700 μL 5 μM thioflavin-T prepared freshly in 50 mM glycine-NaOH (pH 8.5) buffer. The maximum fluorescence intensity of these mixtures was measured at λ_{em} 484 ± 5 nm, with preset λ_{ex} 435 nm. None of the inhibitor compounds showed fluorescence intensity in this region. For the purposes of a screening assay, the fibril signal generated under the conditions of the assay in the presence of 1% DMSO (solvent control) and absence of compound is taken as 100%. The EC_{50} values of potent compounds were determined as described earlier.[13]

Atomic force microscopy of fibrils: The morphology of the incubated peptide samples were studied using atomic force microscopy (AFM).[36,37] Aliquots (2 μL) were spotted on freshly cleaved mica sheets and air dried. The buffer salts were washed off with deionized H_2O. AFM was carried out using a Quesant Q-Scope 250 microscope in non-contact mode.

Assay for inhibition of Aβ oligomer assembly: Biotinyl-$A\beta_{1-42}$, stored as a 1 mg mL^{-1} solution in HFIP at −75 °C, was dried and treated with neat trifluoroacetic acid for 10 min at room temperature to disaggregate the peptide and dissolved to 500 nM (50×) in DMSO as described.[26,27] Peptide (2 μL) was dispensed into each well of a polypropylene 96-well plate, and PBS (100 μL) containing the desired concentration of test compound and 1% DMSO were added to initiate oligomer formation at room temperature. After 30 min, 0.3% *v/v* Tween 20 (50 μL) was added to stop oligomer assembly. This mixture (50 μL) was then assayed for oligomer content by single-site streptavidin-based assay.

Biotinyl-$A\beta_{1-42}$ single-site streptavidin-based assay for determination of inhibitor activity in Aβ oligomer formation:[26,27] NA (50 μL, 1 μg mL^{-1}) in 10 mM NaPi (pH 7.5) was coated per well overnight at 4 °C on Costar 9018 high-binding ELISA plates sealed with adhesive plastic film. The plates were blocked by the addition of 200 μL phosphate-buffered saline (PBS; 10 mM sodium phosphate [pH 7.5], 150 mM NaCl, 0.1% *v/v* Tween 20) at room temperature for 1–2 h and stored at 4 °C. In the assay after removal of the blocking solution, a sample containing a mixture of oligomers and monomers of biotinylated peptide (50 μL containing up to 10 nM Aβ) was bound for 2 h at room temperature. The wells were washed three times with TBST (20 mM Tris-HCl [pH 7.5], 34 mM NaCl, and 0.1% *v/v* Tween 20) on a Biotek EL×50 automated plate washer. After washing, SA–HRP (50 μL 1:20000) in PBS + 0.1% *v/v* Tween 20 was added, the plate sealed, and incubation was continued for 1 h at room temperature. The plate was washed again with TBST, 100 μL tetramethylbenzidine/H_2O_2 substrate solution was added, and the plate was incubated at room temperature for 5–10 min. The OD_{450} was determined on a Biotech Synergy HT plate reader after stopping the reaction with 100 μL H_2SO_4 (1% *v/v*). For the purposes of a screening assay, the oligomer signal generated under the conditions of the assay in the presence of 1% DMSO (solvent control) and absence of compound was taken as 100%.

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