Organofluorine Inhibitors of Amyloid Fibrillogenesis†

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ABSTRACT: The design and application of an effective, new class of organofluorine inhibitors of amyloid fibrillogenesis are described. Based on experimental evidence a core structure containing indol-3-yl, trifluoromethyl, hydroxyl, and carboxylic acid ester functions has been designed. Several substituted derivatives of this core structure have been synthesized, using various indole derivatives. While all inhibitor candidates have shown considerable effect (20−70% inhibition) in structure−activity relationship studies (inhibitor/Aβ = 10 ratio), several compounds have demonstrated excellent activity (93−96% inhibition). Using concentration dependence studies, the activity of the most active molecules have been quantified. These inhibitors practically completely block the fibril formation of Aβ_{1-40}, as shown by maximum inhibition values (IC_{max} = 98−100%). The median inhibitor concentration values (IC_{50} = 0.23−0.53 $mol_{inhibitor}/mol_{A\beta}$) demonstrate favorable stoichiometry for the inhibition. The respective elimination of the functional groups from the core structure has resulted in a partial or complete loss of activity, indicating the significant role of each group. Experiments with these derivatives suggest the particular importance of the acidic hydroxyl group during peptide−inhibitor interaction.

Misfolded, amyloid-like protein deposits in cells and tissues are present in many, frequently aging-related human diseases, including Alzheimer's disease (AD[1]). The major constituent of cerebellar amyloid plaques, characteristic in the case of AD, is the amyloid β (Aβ) peptide (*1*−*6*). As both the soluble oligomeric intermediates of Aβ and its polymeric aggregates (amyloid fibrils) are found neurotoxic (*7*−*10*), inhibition of their formation is one of the possible treatment strategies against AD (*11*). Several findings suggest that a conformational transition from random coil/α-helix to β-sheets is responsible for aggregation (*9*−*11*). Structural studies also indicate the special importance of the central region of Aβ amino acid sequence in aggregation (*12*−*16*), possibly as a self-recognition motif (*17*−*19*). As our goal is the design and synthesis of new fibrillogenesis inhibitors, here we focus only on efforts that have been made regarding this potential treatment option. Due to the importance of this problem it has been reviewed extensively (*9*−*11*, *20*−*22*). Over the years many chemical inhibitors have been tested, and several of them showed potential promise against Aβ fibrillogenesis. These inhibitors include but are not limited to hystological dyes (e.g. Congo Red, Thioflavin S, Chrysamine G) (*23*), rifampicin, naphthoquinones (*20*), special peptides (*17*−*19*), furan derivatives (*24*), indole derivatives (*25*−*27*), proline derivatives (*28*), and a wide range of other compounds (*29*−*32*). Most recently two natural compounds, namely, curcumin (*33*, *34*) and ferulic acid (*35*), have been reported to exhibit antifibrillogenic activity.

Organofluorine compounds are of exceptional interest in medical applications due to the unique properties of the fluorine atom (*36*). Over the past decade a steady stream of fluorinated drugs brought significant advances into drug development, including steroidal and nonsteroidal antiinflammatory and central nervous system drugs, anticancer agents, and antiviral agents (*36*−*38*). According to recent studies a few organofluorine compounds have already been studied in regard to protein misfolding. Adsorption studies of Aβ solutions (containing monomers and small oligomers) on poly(tetrafluoroethylene) surfaces have shown that the fluorinated surface strongly promoted α-helix re-formation (*39*). A similar effect was found by studying the solution of Aβ in CF_3-group-containing solvents (*40*). Analogues of diflunisal were found to be effective inhibitors in the oligomerization of another amyloid protein, transthyretin (*41*).

Herein, we describe the design and application of a new class of organofluorine inhibitors against amyloid fibrillogenesis. We demonstrate exceptional efficacy of these compounds in the in vitro self-assembly of the major variant of amyloid β, the 40 residue Aβ_{1-40} peptide.

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[1] Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β; NMR, nulear magnetic resonance; ESI-MS, electronspray mass spectrometry; EI−MS, electron impact mass spectrometry; GC−MS, gas chromatograph−mass spectrometer; DMSO, dimethyl sulfoxide; TLC, thin-layer chromatography; THF, tetrahydrofuran; DMF, dimethyl formamide.

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MATERIALS AND METHODS

Materials. Amyloid β ($A\beta_{1-40}$) with purity higher than 95% was purchased from American Peptide Company, Inc. Our ESI-MS (Finnigan LCQ Advantage LC−MS system) analysis indicated the correct mass and lack of any peptide fragments. The inhibitors used in this study were synthesized by literature methods (*42*−*46*). The inhibitors have been purified by flash chromatography, and characterized by ^1H, ^{13}C, ^{19}F NMR spectroscopy using a Varian Innova400, superconducting NMR spectrometer (*36*, *37*) ($CDCl_3$ as solvent) and EI-MS using a Shimadzu QP5050 GC−MS, at 70 eV, with 30 m DB-5 capillary column. In order to propose a binding group, derivatives of inhibitors have been synthesized as described below. Dimethyl sulfoxide and other chemicals were all Sigma-Aldrich products.

Fibril Formation Assays. Assays have been based on literature techniques (*17*−*19*, *47*−*49*). The lyophilized $A\beta_{1-40}$ peptide was first dissolved in dimethyl sulfoxide (DMSO) to a concentration of 40 mg/mL. Subsequently, this solution was diluted into 100 mM phosphate buffer, 150 mM NaCl, 0.05% NaN_3, pH 7.4 to a final concentration of 80−100 μM. The individual peptide concentrations were determined using Micro BCA Protein Assay (Pierce). The potential inhibitors were dissolved in DMSO and added to the Aβ solution in different molar ratios (inhibitor/Aβ = 1 to 50). To the control experiment was added DMSO only. All samples contained the same amount of DMSO, and its total concentration was less than 2% (v/v). This amount did not show any effect on the fibrillogenesis (*18*). After 30 s of vigorous vortexing the solutions were incubated at room temperature for 9 days without further agitation. The fibrils formed were characterized by thioflavin T fluorescence, and high-resolution transmission electron microscopy as described below. The quantitative data obtained are the average of 3−5 parallel experiments.

Thioflavin T Fluorescence Assays. The fluorescence measurements have been carried out using a Jobin-Yvon Spex Fluorolog fluorescence spectrophotometer as described in the literature (*18*, *47*, *48*). The incubated peptide solutions were briefly vortexed before each measurement, and then 10 μL aliquots of the suspended fibrils were withdrawn and added into 2 mL of 5 μM thioflavin T solution in 50 mM glycine−NaOH buffer, pH 8.5 and thoroughly mixed. The fluorescence spectra of these mixtures have been measured using 435 nm (excitation) and 484 nm (emission) wavelengths, respectively. None of the inhibitor compounds used in this study exhibited fluorescence intensity in the above-mentioned wavelength region. The calculated I_{THT} values were based on maximum fluorescence intensities in the 480−485 nm region (emission spectra) after subtracting the background fluorescence of the 5 μM thioflavin T in blank buffer.

Transmission Electron Microscopy. Small aliquots (~5 μL) of the samples (see Fibril Formation Assays above) were withdrawn and added to a 400 mesh, carbon-coated Formvar grid (Electron Microscopy Sciences). After 2 min the excess fluid was blotted off, and 1% (w/v) aqueous uranyl acetate was added to the grid for an additional 2 min. Finally, the excess solution was removed, and the grid was allowed to air-dry. The morphological characterization of the negatively stained fibrils was carried out using a JEOL4000FX high-resolution transmission electron microscope operating at 200 kV (*19*, *49*).

^{19}F NMR Study of the Inhibitor−Aβ Interaction. $A\beta_{1-40}$ solution (150 μL, 100 μM) was prepared in 100 mM phosphate buffer, 150 mM NaCl, 0.05% NaN_3, pH 7.4 (see Fibril Formation Assays) containing inhibitor **12**. The molar ratio of the inhibitor and the peptide was set to 20:1. The obtained solution has been diluted with D_2O to 500 μL and placed into an NMR tube. The ^{19}F NMR spectrum (376 MHz) of the solution was obtained on a Varian Innova400 superconducting NMR spectrometer at ambient temperature (23 °C). Due to the low concentration of the ^{19}F-containing species, 11 000 scans were collected. For comparison, the spectrum of the peptide-free **12** in D_2O was also recorded.

*General Procedure for the Synthesis of 3,3,3-Trifluoro-2-hydroxyl-2-(indol-3-yl)-propionic Acid Ethyl Esters (**1**−**12**).* Indole (0.75 mmol) and ethyl 3,3,3-trifluoropyruvate (1.125 mmol) were dissolved in 3 mL of toluene in a Teflon screw cap pressure tube, and 500 mg of K-10 montmorillonite was added. The reaction mixture was immersed into an oil bath preheated to 60 °C. The reaction mixture was stirred by magnetic stirrer, and the progress of the reaction was monitored by TLC (CH_2Cl_2 eluent). After satisfactory conversion, the product was separated from the catalyst by filtration. The solvent and excess TFP were removed in vacuo. The products have been isolated as crystals or oils and purified by flash chromatography.

*General Procedure for the Synthesis of 1-(Indol-3-yl)-2,2,2-trifluoroethanols (**13**−**15**).* Indole (2 mmol) was dissolved in 10 mL of ether and placed in a round-bottom flask equipped with a dry tube. The solution was stirred at 0 °C for 5 min, and then trifluoroacetic acid anhydride (2.1 mmol) was added dropwise. After the complete addition of trifluoroacetic acid anhydride, the mixture was stirred at room temperature for 30 min. The progress of the reaction was monitored by TLC. When the reaction was complete, the solvent and the excess reactant were removed in vacuo. The product was used in the next step without further purification. The crude 3-trifluoroacetyl indole was dissolved in 5 mL of a 1:1 mixture of methanol/THF and placed into a round-bottom flask. The mixture was cooled to 0 °C, $NaBH_4$ (1 mmol) was added slowly, the reaction mixture was stirred at 0 °C, and the progress was monitored by TLC. After completion, the solvent mixture was removed in vacuo. The obtained product was dissolved in CH_2Cl_2, the inorganic salts were removed by filtration, and the solvent was evaporated. The crude product was purified by flash chromatography.

*General Procedure for the Synthesis of 3,3,3-Trifluoro-2-(indol-3-yl)propionic Acid Ethyl Esters (**16**−**18**).* 3,3,3-Trifluoro-2-hydroxy-2-(3-indolyl)propionic acid ethyl ester (1 mmol) was dissolved in 10 mL dry DMF, and the solution was placed in a round-bottom flask equipped with reflux condenser and dry tube. The mixture was cooled to 4 °C, and then $SOCl_2$ (1.5 mmol) was added dropwise at this temperature. After the $SOCl_2$ addition was complete, the reaction mixture was stirred for an additional 1.5 h, then $NaBH_4$ (2 mmol) was added carefully, and the mixture was stirred for another 1 h at 5−10 °C. Then the reaction mixture was poured into 25 mL of 50% NH_4Cl solution, and the product was extracted with ethyl acetate. The crude products have been purified by flash chromatography.

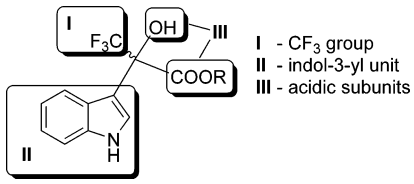


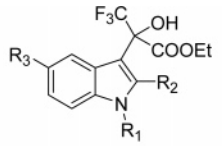
FIGURE 1: Core structure of the proposed new class of organofluorine inhibitors.

General Procedure for the Synthesis of α*-Hydroxy-*α*-(indole-3-yl)acetic Acid Ethyl Esters (**19**−**21**).* Indole (1.0 mmol) and ethyl glyoxylate (1.2 mmol) with 5 mL of 1 M aqueous NaH_2PO_4−Na_2HPO_4 solution were placed into a glass reaction vessel. The mixture was stirred at room temperature for 4 h. The progress was monitored by TLC and GC−MS. After the reaction was complete, 10 mL of saturated $NaHCO_3$ was added and the products were extracted with CH_2Cl_2. The crude products have been purified by flash chromatography.

RESULTS

Experimental Design of the Proposed Organofluorine Inhibitors. Small organic molecules are known to efficiently regulate protein−protein interactions and are proposed to be good candidates to interfere with the aggregation of Aβ (*50*, *51*). For example, in a recent study one such compound has shown promising features in Phase III clinical studies in humans (*28*). The structure determination of amyloid peptides has been the target of numerous studies. Due to the difficulties of growing well-diffracting crystals, several different methods have been necessarily applied to provide structural information (*11*−*14*). Recently, crystals of Aβ fibrils have been produced and characterized by X-ray diffraction using a sequence-designed polypeptide (*15*). Despite the extensive efforts, however, unambiguous, complete structure is not yet available. Accordingly, our inhibitor design is experimental and leans on available information regarding both structural studies and Aβ peptide−small molecule interactions. Based on literature preliminaries the following observations serve as the theoretical basis for the design of a new class of antifibrillogenic compounds:

(i) Fluorinated, especially CF_3-containing, solvents (2,2,2-trifluoroethanol, 1,1,1,3,3,3-hexafluoro-2-propanol) are reported to stabilize the structure of Aβ monomers (*40*). The authors have observed that while ethanol and 2-propanol have exhibited reasonable stabilizing effects only in enormous concentrations (75% concentration, 26% α-helix), the fluorinated derivatives have been significantly more effective stabilizers in remarkably lower concentrations (10% concentration, 48% α-helix). (ii) Indole derivatives, especially carboxylic acid derivatives, have also been reported to inhibit conformational transition, aggregation, and neurotoxicity (*25*−*27*). Melatonin and OXIGON are the two most prominent examples. (iii) The introduction of fluorine atoms is known to highly increase permeability of drugs to the blood−brain barrier (*36*−*38*). Many organofluorine drugs contain the CF_3 group since this group is stable under metabolic conditions (*36*−*38*). In our approach, we combine these findings. Our method is based on the synthesis of trifluoro-indolyl-hydroxyl-propionic acid derivatives (Figure 1). This core structure incorporates the above-mentioned predictably important functional groups. Only a small group of such compounds are known, partially from our own works (*42*−*46*).



	R_1	R_2	R_3
1	H	H	H
2	CH_3	CH_3	H
3	H	H	CH_3
4	CH_3	H	H
5	H	CH_3	H
6	H	Ph	H
7	H	H	OCH_3
8	H	H	COOMe
9	H	H	F
10	H	H	Cl
11	H	H	Br
12	H	H	I

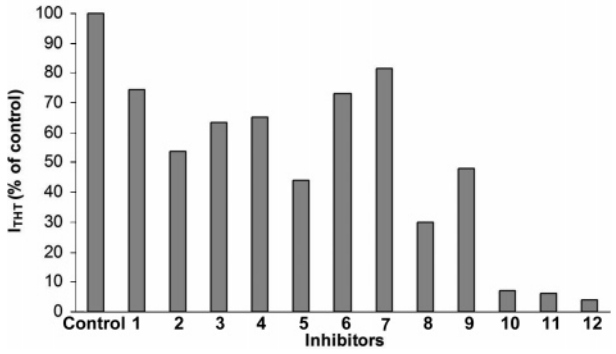


FIGURE 2: Inhibition of Aβ_{1-40} fibrillogenesis by trifluoro-hydroxyl-indolyl-propionic acid esters at $mol_{inhibitor}/mol_{peptide} = 10$ stoichiometry. THT fluorescence intensities (I_{THT}) are normalized to that of the inhibitor-free Aβ_{1-40} sample (control).

Effect of Inhibitor Structure and Concentration on the Antifibrillogenic Activity. As a first step, we have synthesized a wide variety of proposed inhibitors by Friedel−Crafts hydroxyalkylation of indole derivatives with ethyl trifluoropyruvate (*45*, *46*). Then the effect of these compounds on the fibril formation of Aβ has been tested. Fibrillogenesis assays have been carried out using well-defined standard techniques (*17*−*19*, *47*−*49*). Fluorescence spectroscopy of fibril-specific thioflavine T dye has been used for quantitative determination of the amount of fibrils formed in the presence and absence of inhibitors (*6*, *14*). For initial screening, inhibitors have been used in 10-fold molar excess as it was comparable to related studies. In separate investigations we have observed that the amount of fibrils formed from Aβ in inibitor-free environment has shown continuous increase until the eighth day of incubation. After that, intensities turned to saturation. As such, spectroscopic characterizations have been carried out after 9 days of incubation at room temperature when fluorescence intensities of inhibitor-free samples reached a plateau according to our preliminary kinetic studies. The structure of inhibitors and their effect on Aβ_{1-40} fibril formation are illustrated in Figure 2.

As shown, all studied compounds inhibited the fibril formation process, although to different extents. While most molecules have shown considerable effect (20−70% inhibition), several compounds (**10**−**12**) have demonstrated excellent inhibition. In the latter cases, fibril formation is almost completely suppressed; percentile values are between 93%

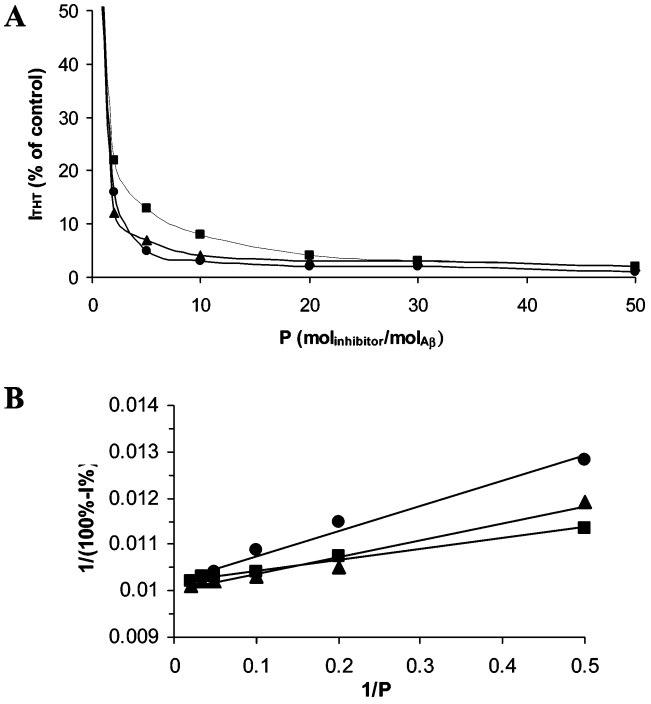


FIGURE 3: (A) Effect of inhibitor/Aβ molar ratios on the amount of fibrils formed in the presence of selected trifluoro-hydroxyl-indolyl-propionic acid esters (**10**−**12**) in comparison to the inhibitor-free Aβ_{1-40} sample (control) based on their thioflavin T fluorescence intensities. (B) Double reciprocal plot of the data for determination of IC_{max} and IC_{50} values (● , inhibited by **10**; ■, inhibited by **11**; ▲, inhibited by **12**).

and 96% inhibition. These data suggest that the presence of an additional bulkier halogen substituent on the carbocylic ring promotes inhibition.

Screening of all four halogens in this position suggests that the size of halogen substituents contributes to the inhibitor effect. Besides the effect of the molecular structure of inhibitor candidates, fibrillogenesis inhibition is known to be a dose-dependent process. As such, the effect of inhibitor concentration on the fibril formation process has been determined at constant Aβ concentration (100 μM). Based on the results of the above-mentioned structure−activity relationship studies (Figure 2) three inhibitors (**10**−**12**) have been selected for these investigations (Figure 3).

Figure 3A illustrates normalized thioflavin T fluorescence intensities as a function of inhibitor/Aβ molar ratio. Fluorescence intensity vs molar ratio functions can be used to determine the relative potency of inhibitors using a simple equation, which is similar to the analysis of the Michaelis−Menten kinetics or ligand binding to macromolecules (Figure 3B) (*17*−*19*),

$$I_{THT} = 100 - \frac{IC_{max}\,P}{IC_{50} + P}$$

where I_{THT} is the fluorescence intensity of the inhibitor-containing sample expressed as a percentage of control, P is the inhibitor/Aβ molar ratio, IC_{50} is the median inhibitor constant, and IC_{max} is the maximum inhibition.

The calculated IC_{50} and IC_{max} values for each inhibitor are tabulated in Table 1.

According to the data IC_{max} values are close to 100%, which indicates that these inhibitors practically completely

Table 1: Summary of Inhibition of Aβ Fibrillogenesis by Selected Small Molecules (100 μM Aβ_{1-40})

inhibitor	IC_{50} ($mol_{inhibitor}/mol_{A\beta}$)	IC_{max} (%)
10	0.53	98
11	0.23	98
12	0.36	100

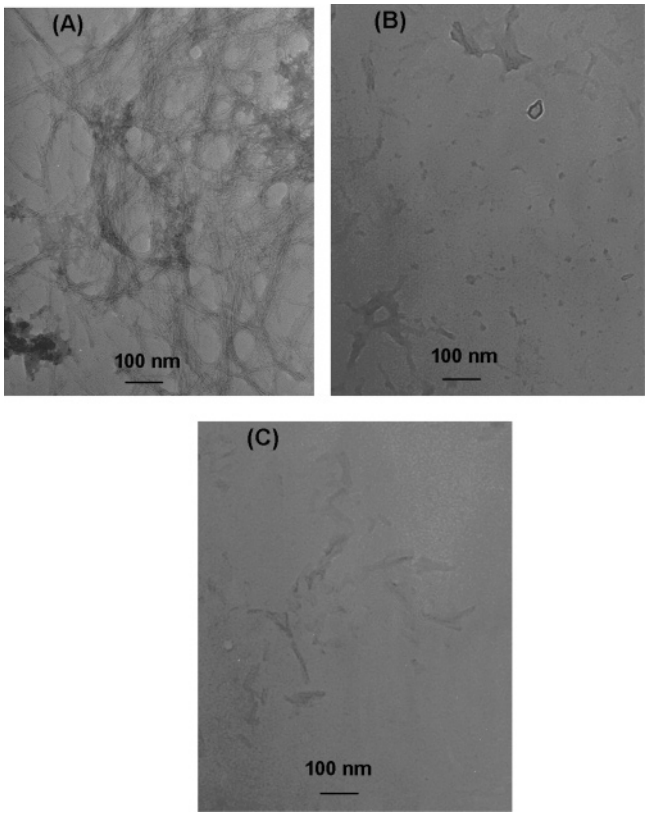


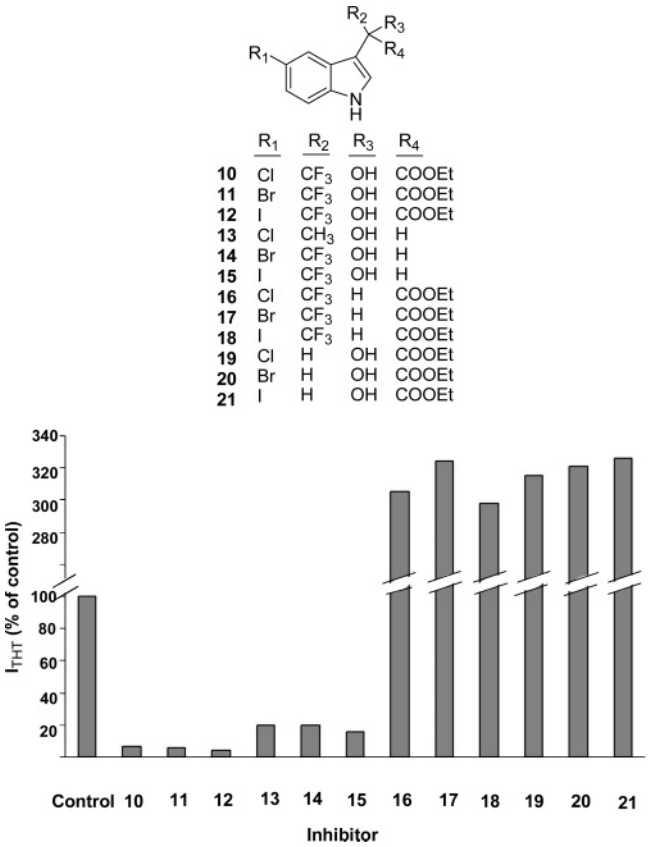
FIGURE 4: Representative transmission electron micrographs indicating the inhibition of Aβ_{1-40} fibrillogenesis by compounds **11** and **12** after 9 days of incubation at $mol_{inhibitor}/mol_{peptide} = 50$ stoichiometry. (A) TEM image of Aβ_{1-40} fibrils formed without any added inhibitor compound. (B) TEM image of Aβ_{1-40} peptide incubated with compound **11**. (C) TEM image of Aβ_{1-40} peptide incubated with compound **12**.

block fibril formation of Aβ_{1-40}. Performances of the inhibitors are very similar; differences induced by individual halogen atoms are minimal. The IC_{50} values unambiguously demonstrate very favorable stoichiometry for inhibition. These numbers clearly indicate that our new organofluorine compounds demonstrate excellent inhibitor effect.

Although thioflavin T fluorescence is a widely accepted assay for quantitative description of antifibrillogenic activity for Aβ fibrillogenesis inhibitors, transmission electron microscopy (TEM) has also been used to confirm our results (Figure 4).

TEM micrographs provide strong support for the data shown above. They indicate significant difference between inhibitor-free Aβ control sample and samples incubated with inhibitors **11** and **12**, respectively. While inhibitor-free peptide formed the expected network of long fibers, complete lack of such aggregates can be observed in the presence of both **11** and **12**.

At this level of the study it is premature to provide a detailed mechanistic picture for the inhibitor effect. As our inhibitors contain fluorine,[19]F NMR spectroscopy is a



	R_1	R_2	R_3	R_4
10	Cl	CF_3	OH	COOEt
11	Br	CF_3	OH	COOEt
12	I	CF_3	OH	COOEt
13	Cl	CH_3	OH	H
14	Br	CF_3	OH	H
15	I	CF_3	OH	H
16	Cl	CF_3	H	COOEt
17	Br	CF_3	H	COOEt
18	I	CF_3	H	COOEt
19	Cl	H	OH	COOEt
20	Br	H	OH	COOEt
21	I	H	OH	COOEt

FIGURE 5: Inhibition of Aβ_{1-40} fibrillogenesis by trifluoro-hydroxyl-indolyl-propionic acid esters (**10**−**12**) and their derivatives (**13**−**19**) at $mol_{inhibitor}/mol_{peptide} = 10$ stoichiometry. THT fluorescence intensities (I_{THT}) are normalized to that of the inhibitor-free Aβ_{1-40} sample (control).

convenient method to follow inhibitor−peptide interaction. Our solution NMR studies (data not shown) indicated that the inhibitor candidates act through binding to the peptide.

In order to learn more about the nature of this interaction and describe the importance of the most characteristic functional groups, several derivatives (**13**−**21**) of the most active inhibitors (**10**−**12)** have been synthesized and tested under identical conditions. This strategy included removal of the ester, hydroxyl, and trifluoromethyl groups, respectively. Structures of the derivatives tested and comparative results are illustrated in Figure 5.

The data indicate that removal of the ester group (**13**−**15**) resulted in compounds which are still active inhibitors (~80% inhibition); however, their relative activity is lower than that of the parent compounds (**10**−**12**). Removing the OH (**16**−**18**) or CF_3 groups (**19**−**21**), however, induced a significant change in activity; these molecules rather acted as fibrillogenesis promoters.

DISCUSSION

The above detailed experimental results clearly indicate that the proposed design for the structure of Aβ fibrillogenesis inhibitors is reasonable. Appropriate selection of substituents on the indole part of the core structure resulted in highly effective inhibitors (Figure 2). The most effective compounds (**10**−**12**) performed well in dose-dependent studies exhibiting practically complete blocking of fibril formation in favorable stoichiometry (Figure 3 and Table 1). Experiments with derivatives of **10**−**12** (Figure 5) suggest that each group has a significant role in the inhibitor effect, but CF_3 and OH groups have a crucial function in binding to the peptide. The common OH substituent is known as a group of very weak acidic character. However, introduction of the strongly electron withdrawing CF_3 group (*36*, *38*, *44*, *52*) significantly increases the acidic nature of OH. The COOEt group, also known as an electron withdrawing substituent, further promotes the acidity of the OH. Significant remaining activity of **13**−**15**, lacking COOEt, indicates that CF_3 is the primarily responsible element in activating OH. Without discussing the nature of inhibitor−Aβ_{1-40} interaction in detail, we suggest that the hydroxyl group of acidic character plays a key role in binding to the peptide possibly to one or both lysine residues (16 and 28). If this acidic hydroxyl group is removed from the molecules, their inhibitor activity completely disappears. A similar effect has been resulted by the removal of the trifluoromethyl group (Figure 5).

Our suggestion is supported by a relatively large number of inhibitors possessing acidic substituents, such as phenolic hydroxyl (−Ph−OH), and carboxyl (−COOH) groups. The most prominent examples are curcumin, OXIGON, ibuprofen, naproxen, and ferulic acid (*20*−*30*). In extended studies with apomorphine and its derivatives, a similar effect was observed. The parent compound having phenolic OH substituents was found to be an effective inhibitor; however, the complete or partial blocking of the acidic phenolic hydroxyl groups resulted in nearly complete loss of activity (*53*). Further support is provided to this interpretation by recent X-ray studies (*15*) where the continuous attachment of the basic and acidic residues in a bricklike arrangement is clearly demonstrated. The provided structure suggests that intrinsic acid−base interaction of the residues plays a role in the self-assembly of the peptide. Our proposed inhibitors are probably replacing the acidic residues of the peptide and inhibit the self-assembly.

In conclusion, we described the design and application of an unprecedented new class of organofluorine Aβ_{1-40} fibrillogenesis inhibitors. The in vitro studies of the proposed compounds demonstrated that three of these molecules are exceptionally effective inhibitors of Aβ self-assembly. Application of these new compounds carries the promise of their becoming potential therapeutics against Alzheimer's disease, and their use may be extended to other protein misfolding disorders, as well.

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SUPPORTING INFORMATION AVAILABLE

Spectral characterization of the compounds used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting Information

for **"**Organofluorine Inhibitors of Amyloid Fibrillogenesis"

by Marianna Török, Mohammed Abid, Shilpa C. Mhadgut, Béla Török

(Spectral characterization of the compounds used in this study)

*3, 3, 3-Trifluoro-2-hydroxy-2-(indol-3-yl)-propionic acid ethyl ester (**1**)* — m.p. 70.5-71.8 ^{o}C, **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.25 (bs, 1H, NH), 7.89 (d, *J*=8.3 Hz, 1H, Ar), 7.45 (d, *J*=2.3 Hz, 1H, Ar), 7.35 (dd, *J*=7.99, 1.19 Hz,1H, Ar), 7.21 (dd, *J*= 7.19, 1.19 Hz, 1H, Ar), δ 7.14 (ddd, *J*=7.19, 1.19 Hz, 1H, Ar), 4.44 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 4.39 (s, 1H, OH), 4.34 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 1.35 (td, *J*=7.19 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 169.6, 136.5, 125.3, 125.1, 124.5, 122.9, 122.3, 121.4, 120.7, 111.5, 108.9, 64.4, 14.1; **^{19}F NMR** (376.19 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) -77.15 (s, 3F); **MS**-$C_{13}H_{12}F_3NO_3$ (287), m/z (%): 287 (M^+, 33), 214 (100), 144 (65), 117 (70), 89 (30).

*3,3,3-Trifluoro-2-hydroxy-2-(1,2-dimethyl-indol-3-yl)-propio-nic acid ethyl ester (**2**)* — m.p 83-84.5 ^{o}C, **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 7.77 (d, *J* =7.99 Hz, 1H, Ar), 7.26 (d, *J*=8.3 Hz, 1H, Ar), 7.17 (ddd, *J*=6.7, 1.1 Hz, 1H, Ar), 7.08 (ddd, *J*=6.7, 1.1 Hz, 1H, Ar), 4.41 (dq, *J*=7.1, 3.1 Hz, 1H, CH_2), 4.39 (s, 1H, OH), 4.36 (dq, *J*=7.1, 3.1 Hz, 1H, CH_2), 3.65 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 1.35 (td, *J*=7.2, 3H, CH_3); **^{13}C NMR** (100.54 MHz, $CDCl_3$), δ (ppm) 169.5, 137.1, 136.6, 129.2, 125.9, 124.8, 122.7, 121.4, 120.2, 109.1, 103.7, 63.5, 29.6, 14.1, 11.8; **^{19}F NMR** (376.15 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) -77.01 (s, 3F); **MS**-$C_{15}H_{16}F_3NO_3$ (315), m/z (%): 315 (M^+, 33), 242 (100), 172 (66), 145 (85).

*3,3,3-Trifluoro-2-hydroxy-2-(5-methyl-indol-3-yl)-propionic acid ethyl ester (**3**)* — (m.p. 75.2-76.5 ^{o}C; **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.16 (bs, 1H, NH), 7.66 (s, 1H, Ar), 7.40 (d, *J*=2.3 Hz, 1H, Ar), 7.24 (d, *J*=8.3 Hz, 1H, Ar), 7.03 (dd, *J*=8.3, 1.5 Hz, 1H, Ar), 4.43 (dq, *J*=7.19, 3.6 Hz, 1H, CH_2), 4.39 (s, 1H, OH), 4.34 (dq, *J*=7.19, 3.5 Hz, 1H, CH_2), 2.43 (s, 3H, CH_3), 1.35 (td, *J*=7.19 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 169.6, 151.7, 134.8, 130.0, 125.6, 124.5, 123.4, 122.3, 120.9, 111.1, 108.4, 64.3, 21.8, 14.0; **^{19}F NMR** (376.19 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) -77.12 (s, 3F); **MS**-$C_{14}H_{14}F_3NO_3$ (301), m/z (%): 301 (M^+, 11), 130 (100).

*3,3,3-Trifluoro-2-hydroxy-2-(1-methyl-indol-3-yl)-propionic acid ethyl ester (**4**)* — m.p 61-62.2 ^{o}C, **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 7.87 (d, *J*=8.3 Hz, 1H, Ar), 7.31 (d, *J*=2.7 Hz, 1H, Ar), 7.29 (dd, *J*=1.5 Hz, 1H, Ar), 7.24 (ddd, *J*=6.7, 0.7 Hz, 1H, Ar), 7.14 (ddd, *J*=6.7, 1.1 Hz, 1H.Ar), 4.45 (dq, *J*=7.19, 3.5 Hz, 1H, CH_2), 4.39 (s, 1H, OH), 4.32 (dq, *J*=6.7, 3.5 Hz, 1H, CH_2), 3.77 (s, 3H, CH_3), 1.35 (td, *J*=7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.54 MHz, $CDCl_3$), δ (ppm) 169.6, 137.4, 129, 125.9, 125.1, 122.4, 121.4, 120.3, 109.7, 107.0, 64.3, 33.2, 14.1; **^{19}F NMR** (376.15 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) -77.17 (s, 3F); **MS**-$C_{14}H_{14}F_3NO_3$ (301), m/z (%): 301 (M^+, 25), 228 (100), 158 (60), 131 (80).

*3,3,3-Trifluoro-2-hydroxy-2-(2-methyl-indol-3-yl)-propionic acid ethyl ester (**5**)* — m.p. 67-68.5 ^{o}C, **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 7.99 (bs, 1H, NH), 7.79 (d, *J*=7.9 Hz, 1H, Ar), 7.24 (dq, *J*=6.7, 0.7 Hz, 1H, Ar), 7.12 (ddd, *J*=7.1, 1.1 Hz, 1H, Ar), 7.08 (ddd, *J*=6.7, 1.1 Hz, 1H.Ar), 4.43 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 4.34 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 3.95 (s, 1H, OH), 2.51 (s, 3H, CH_3), 1.33 (t, *J*=7.19 Hz, 3H, CH_3), **^{13}C NMR** (100.54 MHz, $CDCl_3$), δ (ppm) 169.5, 135.4, 134.8, 127, 125.5, 122.7, 121.8, 120.7, 120.4, 110.4, 104.1, 63.77, 14.4, 14.0; **^{19}F NMR** (376.15 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) -77.91 (s, 3F); **MS**-$C_{14}H_{14}F_3NO_3$ (301), m/z (%): 301 (M^+, 30), 228 (100), 158 (66), 131 (90).

*3,3,3-Trifluoro-2-hydroxy-2-(2-phenyl-indol-3-yl)-propionic acid ethyl ester (**6**)* — m.p 145.0-147.0 ^{o}C; **^{1}H NMR** (399.81 MHz,$CDCl_3$), δ (ppm) 8.14 (bs, 1H, NH), 8.04 (d, *J*=8.0 Hz,1H, Ar), 7.43 (m, 5H, Ar), 7.32 (td, *J*=8.0, 1.2 Hz,1H, Ar), 7.23 (ddd, *J*=5.6, 1.2 Hz, 1H, Ar), 7.18 (ddd, *J*=7.2, 1.5 Hz, 1H, Ar), 3.88 (dq, *J*=10.4, 7.2 Hz,1H, CH_2), 3.72 (s,1H, OH), 3.50 (dq, *J*=14.4, 7.2 Hz, 1H, CH_2), 1.08 (t, *J*=7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.54 MHz, $CDCl_3$), δ (ppm) 168.7, 138.0, 135.3, 132.7, 130.5, 129.2, 128.1, 126.7, 125.4, 123.0, 122.6, 122.5, 122.9, 110.8, 106.2, 63.1, 13.7; **^{19}F NMR** (376.19 MHz, $CDCl_3$, $CFCl_3$-Ref), δ (ppm) -75.63 (s, 3F); **MS**-$C_{19}H_{16}F_3NO_3$ (363), m/z (%): 363 (M^+, 15), 290 (66), 220 (50), 193 (100).

*3,3,3-Trifluoro-2-hydroxy-2-(5-methoxy-indol-3-yl)-propionic acid ethyl ester (**7**)* — m.p. 74.5-75.8 ^{o}C; **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.25 (bs, 1H, NH), 7.40 (d, *J*=2.8Hz, 1H, Ar), 7.35 (d, *J*=2.4 Hz, 1H, Ar), 7.22 (d, *J*=8.8 Hz, 1H, Ar), 6.87 (dd, *J*=8.8, 2.4 Hz, 1H, Ar), 4.45 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 4.40 (s, 1H, OH), 4.34 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 3.83 (s, 3H, CH_3), 1.34 (td, *J*=7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 169.6, 154.7, 131.6, 125.9, 125.2, 125, 122.3, 113.4, 112.2, 108.5, 103, 64.4, 56.0, 14.2; **^{19}F NMR** (376.15 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) -77.15 (s, 3F); **MS**-$C_{14}H_{14}F_3NO_4$(317), m/z (%): 317 (M^+, 25), 244 (95), 270 (65), 147 (100).

*3,3,3-Trifluoro-2-hydroxy-2-(5-methoxycarbonyl-indol-3-yl)-propionic acid ethyl ester (**8**)* — **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ(ppm) 8.70 (bs, 1H, NH), 8.68 (s,1H, Ar), 7.90 (dd, *J* = 8.8, 2.0 Hz, 1H, Ar), 7.54 (d, *J* = 2.8 Hz, 1H, Ar), 7.36 (dd, *J* =8.8, 0.4 Hz, 1H, Ar), 4.45 (dq, *J* = 7.2, 3.6 Hz, 1H, CH_2), 4.40 (s, 1H, OH), 4.35 (dq, *J* = 6.4, 3.6 Hz, 1H, CH_2), 3.91 (s, 3H, CH_3), 1.35 (td, *J* = 5.2 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ(ppm) 169.3, 168.3, 167.2, 139.2, 126.2, 124.9, 124.6, 124.1, 122.7, 121.5, 111.3, 110.3, 64.6, 52.1, 14.0; **^{19}F NMR** (376.15 MHz, $CDCl_3$, $CFCl_3$-ref), δ(ppm) -76.03 (s, 3F); **MS**-$C_{15}H_{14}F_3NO_5$ (345), m/z (%): 345 (M^+, 25), 272 (100), 202 (50), 144 (40).

*3,3,3-Trifluoro-2-hydroxy-2-(5-fluoro-indol-3-yl)-propionic acid ethyl ester (**9**)* — **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.28 (bs, 1H, NH), 7.57 (dd, *J* = 10.4, 2.8 Hz,1H, Ar), 7.50 (d, *J* = 2.8 Hz, 1H, Ar), 7.26 (m, 1H, Ar), 6.96 (ddd, *J* = 8.8, 2.4 Hz, 1H, Ar), 4.45 (dq, *J* = 6.8, 3.2 Hz, 1H, CH_2), 4.41 (s, 1H, OH), 4.36 (dq, *J* = 6.8, 3.5 Hz, 1H, CH_2), 1.36 (td, *J* = 7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 169.3, 159.4, 157.1, 133, 126.2, 112.1, 112, 111.6, 111.4, 106.8, 106.6, 64.8, 14.1; **^{19}F NMR** (376.15 MHz, $CDCl_3$, $CFCl_3$-Ref), δ (ppm) -77.487 (s, 3F), -124.251 (m, 1F); **MS**-$C_{13}H_{11}F_4NO_3$ (305), m/z (%): 305 (M^+, 20), 232 (100), 162 (50), 135 (40).

*3,3,3-Trifluoro-2-hydroxy-2-(5-chloro-indol-3-yl)-propionic acid ethyl ester (**10**)* — m.p. 51-52.3 °C; **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.30 (bs, 1H, NH), 7.91 (t, *J*=0.8 Hz, 1H, Ar), 7.50 (d, *J*=2.8 Hz, 1H, Ar), 7.28 (dd, *J*=8.8, 0.8 Hz, 1H, Ar), 7.17 (dd, *J*=6.8, 2.0Hz, 1H, Ar), 4.45 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 4.41 (s, 1H, OH), 4.36 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 1.36 (td, *J*=7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.54 MHz, $CDCl_3$), δ (ppm) 169.3, 134.9, 126.5, 126.3, 125.9, 125, 123.2, 122.1, 121.1, 112.5, 108.3, 64.6, 14.0; **^{19}F NMR** (376.15 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) -77.53 (s, 3F); **MS**-$C_{13}H_{11}F_3ClNO_3$ (321), m/z (%): 321 (M^+, 20), 248 (100), 178 (60), 151 (85).

*3,3,3-Trifluoro-2-hydroxy-2-(5-bromo-indol-3-yl)-propionic acid ethyl ester (**11**)* — m.p. 51-52.5 °C; **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.32 (bs, 1H, NH), 8.07 (t, *J*=0.7 Hz, 1H, Ar), 7.47 (d, *J*= 2.7 Hz, 1H, Ar), 7.29 (dd, *J*= 8.8, 2.0 Hz, 1H, Ar), 7.22 (dd, *J*=8.8, 0.3 Hz, 1H, Ar), 4.45 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 4.39 (s, 1H, OH), 4.36 (dq, *J*=7.2, 3.9 Hz, 1H, CH_2), 1.36 (td, *J*=7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.54 MHz, $CDCl_3$), δ (ppm) 169.3, 135.2, 127.0, 125.9, 125.8, 125, 124.26, 122.1, 114.2, 112.9, 108.7, 64.7, 14.1; **^{19}F NMR** (376.15 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) -77.5 (s, 3F); **MS**-$C_{13}H_{11}F_3BrNO_3$ (367), m/z (%): 367 (M^+, 10), 295 (45), 225 (20), 144 (100).

*3,3,3-Trifluoro-2-hydroxy-2-(5-iodo-indol-3-yl)-propionic acid ethyl ester (**12**)* — m.p. 74.3-76 °C; **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.35 (bs, 1H, NH), 8.27 (s, 1H, Ar), 7.45 (dd, *J*=8.8, 2.0 Hz, 1H, Ar), 7.40 (d, *J*=2.4 Hz, 1H, Ar), 7.11 (dd, *J*=8.8, 0.4 Hz, 1H, Ar), 4.44 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 4.38 (s, 1H, OH), 4.35 (dq, *J*=7.2, 3.6 Hz, 1H, CH_2), 1.36 (td, *J*=7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.54 MHz, $CDCl_3$), δ (ppm) 169.3, 135.6, 131.3, 130.4, 127.4, 125.5, 124.9, 122.1, 113.4, 108.3, 84.5, 64.7, 14.1; **^{19}F NMR** (376.15 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) -77.46 (s, 3F); **MS**-$C_{13}H_{11}F_3INO_3$ (413), m/z (%): 413 (M^+, 33), 340 (60), 270 (20), 144 (100).

*2,2,2-Trifluoro-1-(5-chloro-indole-3-yl) ethanol (**13**)* — **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.37 (bs, 1H, NH), 7.68 (d, *J*=1.6 Hz, 1H, Ar), 7.28 (d, *J*=2.4 Hz, 1H, Ar), 7.25 (dd, *J*=8.4, 0.4 Hz, 1H, Ar), 7.17 (dd, *J*=8.4, 1.9 Hz, 1H, Ar), 5.25 (q, *J*=13.2, 6.4 Hz, 1H, CH), 2.71 (bs, 1H, OH); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 134.6, 126.9, 126.5, 125.9, 125.4, 123.4, 119.1, 112.7, 109.5, 67.8, **^{19}F NMR** (376.19 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) –78.32 (d, 3F); **MS**-$C_{10}H_7F_3ClNO$ (249), m/z (%): 249 (M^+, 90), 180 (100), 152 (30), 117 (50).

*2,2,2-Trifluoro-1-(5-bromo-indole-3-yl) ethanol (**14**)* — **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.33 (bs, 1H, NH), 7.82 (d, *J*=1.6 Hz, 1H, Ar), 7.29 (dd, *J*=8.8, 2.0 Hz, 1H, Ar), 7.22 (d, *J*=2.8 Hz, 1H, Ar), 7.18 (dd, *J*=8.8, 0.8 Hz, 1H, Ar), 5.22 (q, *J*=13.6, 6.8 Hz, 1H, CH), 2.84 (bs, 1H, OH); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 134.9, 127.5, 126.0, 125.3, 123.5, 122.2, 114.0, 113.2, 109.2, 67.8; **^{19}F NMR** (376.19 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) –78.24 (d, 3F); **MS**-$C_{10}H_7F_3BrNO$ (293), m/z (%): 293 (M^+, 80), 224 (100), 144 (30), 117 (50).

*2,2,2 Trifluoro-1-(5-iodo-indole-3-yl) ethanol (**15**)* — **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.31 (bs, 1H, NH), 8.07 (d, *J*=0.8 Hz, 1H, Ar), 7.48 (dd, *J*=8.4, 1.6 Hz, 1H, Ar), 7.27 (d, *J*=2.4 Hz, 1H, Ar), 7.15 (dd, *J*=8.8, 0.4 Hz, 1H, Ar), 5.22 (q, *J*=13.6, 6.4 Hz, 1H, CH), 2.54 (bs, 1H, OH); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 135.3, 131.5, 128.5, 128.4, 124.7, 123.5, 113.5, 109.2, 84.4, 67.8; **^{19}F NMR** (376.19 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) –78.36 (d, 3F); **MS**-$C_{10}H_7F_3INO$ (341), m/z (%): 341 (M^+, 100), 272 (80), 145 (50), 116 (20).

*3,3,3-Trifluoro-2-(5-chloro-indol-3-yl)-propionic acid ethyl ester (**16**)* — **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.35 (bs, 1H, NH), 7.63 (d, *J*=1.5 Hz, 1H, Ar), 7.42 (d, *J*=2.8 Hz, 1H, Ar), 7.30 (dd, *J*=8.7, 0.8 Hz, 1H, Ar), 7.18 (dd, *J*=8.7, 1.9 Hz, 1H, Ar), 4.57 (q, *J*=16.8, 8.4 Hz, 1H, CH), 4.23 (m, 2H, CH_2), 1.26 (t, *J*=6.8 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 154.7, 134.3, 127.7, 126.6, 126.2, 125.1, 123.4, 121.5, 118.5, 112.6, 62.3, 50.4, 14.1; **^{19}F NMR** (376.19 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) –68.47 (d, 3F); **MS**-$C_{13}H_{11}F_3ClNO_2$ (305), m/z (%): 305 (M^+, 40), 232 (100), 197 (20).

3,3,3-Trifluoro-2-(5-bromo-indol-3-yl)-propionic acid ethyl ester (**17**) — m.p. 74.1-75.8 °C; **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.50 (bs, 1H, NH), 7.80 (d, *J*=1.5 Hz, 1H, Ar), 7.28 (m, 3H, Ar), 4.59 (q, *J*=16.8, 8.3 Hz, 1H, CH), 4.28 (m, 2H, CH_2), 1.26 (t, *J*=7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 167.6, 134.7, 128.3, 125.9, 125.8, 122.9, 121.5, 114.9, 113.2, 103.9, 63.8, 62.5, 14.1; **^{19}F NMR** (376.19 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) –68.46 (d, 3F); **MS**-$C_{13}H_{11}F_3BrNO_2$ (349), m/z (%): 349 (M^+, 50), 276 (100), 226 (10), 197 (20).

*3,3,3-Trifluoro-2-(5-iodo-indol-3-yl)-propionic acid ethyl ester (**18**)* — m.p. 80.5-82.1 °C, **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.50 (bs, 1H, NH), 7.99 (s, 1H, Ar), 7.46 (dd, *J*=8.8, 1.5 Hz, 1H, Ar), 7.32 (d, *J*=2.4 Hz, 1H, Ar), 7.13 (dd, *J*=8.4, 0.4 Hz, 1H, Ar), 4.58 (q, *J*=17.1, 8.7 Hz, 1H, CH), 4.23 (m, 2H, CH_2), 1.26 (t, *J*=7.19 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 166.8, 135.1, 131.3, 129.1, 127.8, 125.7, 122.9, 113.7, 103.6, 84.2, 62.4, 54.3, 14.1; **^{19}F NMR** (376.19 MHz, $CDCl_3$, $CFCl_3$-ref), δ (ppm) –68.35 (d, 3F); **MS**-$C_{13}H_{11}F_3INO_2$ (397), m/z (%): 397 (M^+, 60), 324 (100), 197 (70).

(5-Chloro-indole-3-yl)-hydroxy-acetic acid ethyl ester (**19**) — **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.20 (bs, 1H, NH), 7.64 (s, 1H, Ar), 7.23 (d, *J* = 8.7 Hz, 1H, Ar), 7.14 (d, *J* = 2.4 Hz, 1H, Ar), 6.97 (dd, *J* = 8.7, 2.4 Hz, 1H, Ar), 5.40 (m, 1H, CH), 4.13 (m, 2H, CH_2), 3.50 (bs, 1H, OH), 1.17 (t, *J* = 7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 174.0, 135.0, 127.7, 125.9, 125.0, 122.9, 119.0, 112.7, 111.6, 67.3, 62.4, 14.1; **MS**-$C_{12}H_{12}ClNO_3$ (253), m/z (%): 253 (M^+, 20), 180 (100), 152 (30), 117 (50).

(5-Bromo-indole-3-yl)-hydroxy-acetic acid ethyl ester (**20**) — **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.29 (bs, 1H, NH), 7.80 (s, 1H, Ar), 7.25 (d, *J* = 8.3 Hz, 1H, Ar), 7.16 (d, *J* = 2.8 Hz, 1H, Ar), 6.97 (dd, *J* = 8.3, 2.8 Hz, 1H, Ar), 5.37 (m, 1H, CH), 4.17 (m, 2H, CH_2), 3.70 (bs, 1H, OH), 1.18 (t, *J* = 7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 174.0, 135.3, 125.9, 125.9, 125.4, 122.1, 121.9, 113.3, 109.5, 67.3, 62.4, 14.1; **MS**-$C_{12}H_{12}BrNO_3$ (297), m/z (%): 297 (M^+, 10), 224 (100), 195 (40), 117 (60).

(5-Iodo-indole-3-yl)-hydroxy-acetic acid ethyl ester (**21**) — **^{1}H NMR** (399.81 MHz, $CDCl_3$), δ (ppm) 8.35 (bs, 1H, NH), 8.0 (s, 1H, Ar), 7.35 (d, *J* = 7.4 Hz, 1H, Ar), 7.13 (d, *J* = 2.3 Hz, 1H, Ar), 7.03 (dd, *J* = 7.4, 2.3 Hz, 1H, Ar), 5.58 (m, 1H, CH), 4.20 (m, 2H, CH_2), 3.80 (bs, 1H, OH), 1.18 (t, *J* = 7.2 Hz, 3H, CH_3); **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 171.1, 135.7, 131.1, 129.1, 128.4, 126.3, 124.5, 113.7, 108.5, 67.3, 62.5, 14.2; **MS**-$C_{12}H_{12}INO_3$ (347), m/z (%): 347 (M^+, 25), 274 (100), 245 (70), 117 (30).