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Tuning Transthyretin Amyloidosis Inhibition Properties of Iododiflunisal by Combinatorial Engineering of the Nonsalicylic Ring Substitutions

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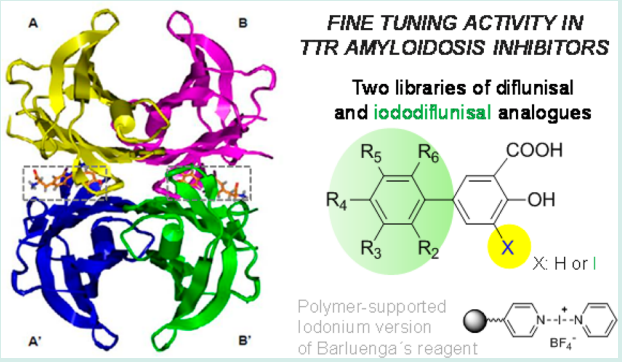
Supporting Information

ABSTRACT: Two series of iododiflunisal and diflunisal analogues have been obtained by using a two step sequential reaction solution-phase parallel synthesis. The synthesis combined an aqueous Suzuki-Miyaura cross-coupling and a mild electrophilic aromatic iodination step using a new polymer-supported iodonium version of Barluenga's reagent. From a selected set of 77 noniodinated and 77 iodinated diflunisal analogues, a subset of good transthyretin amyloid inhibitors has been obtained with improved turbidimetry inhibition constants, high binding affinity to transthyretin, and good selectivity for TTR compared to other thyroxine binding proteins.



KEYWORDS: *transthyretinkinetic stabilizers, TTR amyloidosis inhibitors, solution-phase parallel synthesis, 5-aryl salicylic acid core libraries, iododiflunisal analogues library, polymer-supported iodonium reagent, Barluenga's reagent, aqueous Suzuki-Miyaura reaction*

INTRODUCTION

Transthyretin (TTR) related diseases are a group of rare diseases affecting the peripheral nerves and other vital organs by the formation of amyloid deposits of this protein. They comprise senile systemic amyloidosis (SSA), familial amyloidotic polyneuropathy[1] (FAP), and familial amyloidotic cardiomyopathy (FAC).[2,3]

Many different families of small molecules are known to stabilize TTR effectively thus preventing its aggregation *in vitro*. Within these families, compounds that display *in vitro* transthyretin (TTR) amyloid inhibitory activity usually bind and stabilize the tetrameric form of TTR by mimicking the action of thyroid hormones. These compounds differentially stabilize the native tetrameric structure of TTR over the dissociative transition state, raising the kinetic barrier, imposing kinetic stabilization on the tetramer, preventing TTR amyloidogenesis.[4] Already identified families of inhibitors belong to classes of compounds as diverse as tetrahydroquinolines, dihydropyridines, benzodiazepines, phenoxazines, stilbenes, and benzoxazoles.[5] From all of them, only two TTR tetramer stabilizers, tafamidis and the FDA registered NSAID, diflunisal, were shown to slow down disease progression of FAP. Tafamidis (Vyndaqel) is a newly developed[6] small molecule having a benzoxazole scaffold that binds selectively to TTR (K_{d1} = 2 nM, K_{d2} = 154 nM) and stabilizes the tetrameric structures of both wild-type and variant TTRs. Tafamidis was approved by the European Medicines Agency in 2011 for the treatment of early stage (stage I) FAP and by the Japanese Pharmaceuticals and Medical Devices Agency in 2013 for the treatment of FAP (any stage).[7] Alternatively, the FDA registered NSAID diflunisal has spawned a surge of interest and is currently being tested in clinical trials in FAP patients for its efficacy to ameliorate peripheral neuropathy resulting from TTR depositions.[8]

In our hands the biological properties of diflunisal were improved by iodination on its salicylic moiety.[9] The rational was to imitate the unique structural feature of thyroid hormones of presenting iodine atoms because these hormones are the natural ligands of the TTR binding site where halogen binding pockets have been defined.[10] The strategy rendered one of our

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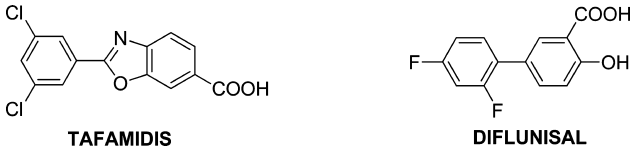


lead compounds, iododiflunisal (IDIF), a small molecule with negligible thyromimetic activity (affinity to thyroid hormone nuclear receptors) and cyclooxygenase-1 (COX-1) activities (see Annex II in the Supporting Information). A first optimization of our lead compound iododiflunisal (IDIF) was conducted by systematic modifications on the functional groups of its salicylic ring.[11] Recently, we have also shown[12] that halogenation of diflunisal gradually improves binding up to one order of magnitude from fluorine (see FDIF in Figure 1) to

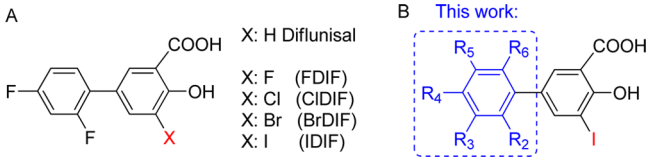


Figure 1. (A) Our lead compound iododiflunisal (IDIF) and other halogenated diflunisal derivatives. (B) Iododiflunisal modifications proposed in this work on the nonsalicylic acid ring (inside the dashed line).

iodine substitution (see IDIF in Figure 1) through interactions that can be interpreted as a suboptimal halogen bonding (XB) or as rather optimized van der Waals contacts or as a mixture of both.[13] In this work, we report a close examination of the substitution pattern of the 2,4-difluorophenyl unit on iododiflunisal in order to fine-tune the amyloidogenic inhibition properties of iododiflunisal (Figure 1).

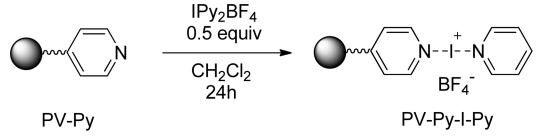
RESULTS AND DISCUSSION

Toward this goal, a solution-phase parallel synthesis of two libraries of *in vitro* fibrillogenesis inhibitory compounds was envisaged to explore the chemical space around this ring by a two steps retrosynthetic scheme (Figure 2).

To conduct the first step of the synthetic route, this is the preparation of the first library of diflunisal analogues, an aqueous Suzuki-Miyaura type of reaction was tested for the diflunisal analogues by adapting already known procedures.[14a,b] The Suzuki-Beletskaya cross-coupling reaction in water at room temperature using $Pd(OAc)_2$ proved to be superior than the organic solvent based alternatives already described for these compounds[14c,d] because of its milder conditions and avoidance of a saponification step (see Scheme S1 in Supporting Information).[15]

The main feature for the preparation of the second library was the use of Barluenga's reagent, the iodonium complex *bis*(pyridine)iodonium(I) tetrafluoroborate (IPy_2BF_4), a versatile and mild reagent to perform a wide range of reactions.[16] Toward upgrading the application of this versatile reagent for combinatorial chemistry procedures,[17,18] here we describe the preparation and the first example of its use in parallel synthesis. Briefly, after testing different immobilization materials and methods,[19] a simple method such as suspending a slurry of poly(4-vinylpyridine) beads (PV-Py) in a solution of Barluenga's reagent afforded the functional material with high iodonium load (PV-Py-I-Py in Scheme 1).

Scheme 1. Synthesis of the Polymer Supported Iodonium Reagent



The reactivity pattern of the polymer supported iodonium reagent against aromatic standard substrates does not significantly deviate from its soluble counterpart, but the polymeric version facilitates product purification and avoids the usual aqueous thiosulfate extractions required. The library of diflunisal analogues (5-aryl salicylic acids) in the first step readily reacted in a second reaction step with either soluble IPy_2BF_4 reagent or with the polymer-supported version of the reagent. In this latter case, the reaction also proceeded satisfactorily in ethanol or methanol rendering clear crude reaction mixtures because the excess of supported reagent do not readily decomposes into iodine as does the soluble counterpart which always yields dark-brown solutions.

The two steps in Scheme 2 were used to obtain a discrete library of both diflunisal and iododiflunisal analogues by parallel synthesis. First, 108 commercial aryl boronic acids were selected, having in common a single six membered aromatic ring and showing a variety of mono-, di-, and trisubstitution patterns resulting from the combination of groups such as fluoro, chloro, bromo, methyl, trifluoromethyl, *tert*-butyl, ethyl, isopropyl, phenyl, benzyl, silyl, methoxy, benzyloxy, phenoxy, carboxyl, hydroxyl, amino, and acetamido (see Figure S3 in the Supporting Information).

Appropriate amounts of $Pd(OAc)_2$ and individual boronic acids were weighed, and aliquots of aqueous and dioxane solutions of the base (Na_2CO_3) and 5-iodosalicylic acid, respectively, were delivered and reacted in a Multiple Organic Synthesizer. By including duplicated reactions of diflunisal

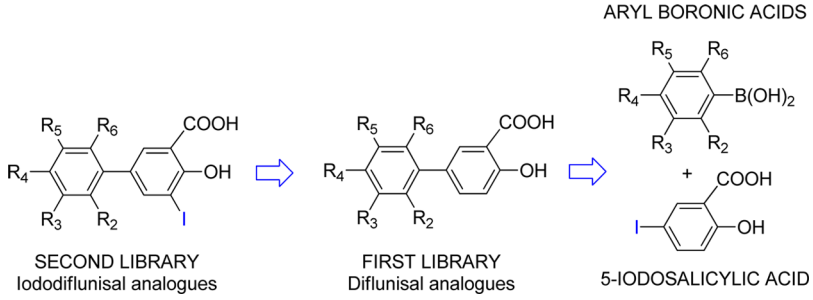


Figure 2. Retrosynthetic analysis of the libraries.

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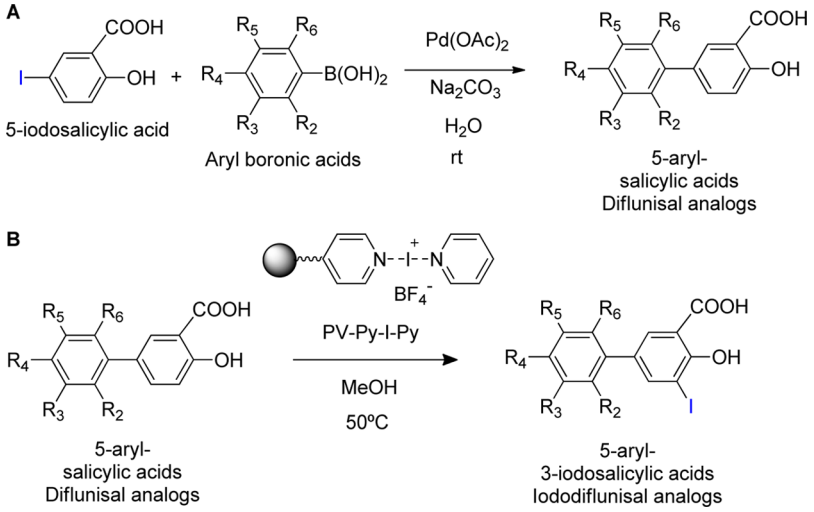
Scheme 2. (A) Aqueous Suzuki-Miyaura Cross-Coupling Reaction and (B) Electrophilic Aromatic Iodination Reaction Effected by the Polymer-Supported Reagent (PV-Py-I-Py)



synthesis spread through the library series, accuracy and reproducibility of the instrument were thus tested.

HPLC analyses of the 108 compounds of the library showed that 77 compounds were obtained with conversions in the range of 80−100%. After discarding the other 31 low yielding reactions, half of the mass of each of the 77 crude reaction products was treated with an excess of the polymer-supported iodonium reagent in methanol using the synthesizer. Most of the HPLC conversions of the iodination reactions were within the range of 60−80% (see Table S3 in the Supporting Information).

The biological activity of the products of such library composed of 77 noniodinated and 77 iodinated analogues has been examined by testing their fibrillogenesis inhibition properties on a high throughput screening turbidimetric kinetic assay that monitors TTR acid-induced fibrillogenesis[20] (for full data see Table S4 in the Supporting Information).

Structure−Activity Relationships. Several trends can be extracted from inspection of the IC_{50} values of all the tested compounds (see the Supporting Information): (a) Monosubstituted compounds at the nonsalicylic ring (with substituents such as halogens, acids, alcohols, ethers, nitro derivatives, amines, and acetamide, among others, at different positions of the residue on the nonsalicylic ring) are in general bad or poor inhibitors. In those compounds iodination has not a significant effect, except for compound **10** selected in the group of the 10 top inhibitors in the screening (Table 1). The carboxylic acid function in the nonsalicylic ring of **10** may result in a different binding mode than the reference diflunisal and iododiflunisal.[21] (b) Disubstituted compounds in positions 3′,5′- and 2′,4′- are moderate inhibitors, but iodination consistently improves their inhibition properties (except for the 3′,5′-dichlorinated analogues **8** and **4** that are already good inhibitors, in those compounds iodination has essentially no additional effect). The 3′,5′-dimethylated compound **4** and its iodinated derivative **5**, the 3′,5′-dichlorinated compound **8**, and the reference iododiflunisal (**1**, IDIF) are among the 10 best inhibitors selected from this screening (Table 1). (c) Disubstituted compounds in positions 2′,5′- are in general poor or moderate inhibitors, and iodination does not improve their inhibition properties. However, the 2′,5′-difluorinated compound **2** and its corresponding iodinated derivative **3** are both good inhibitors and are selected in the group of the 10 top inhibitors in the screening (Table 1). (d) Methoxy substitutions on the nonsalicylic ring render poor inhibitors, with no improvement upon iodination. If an halogen substitution is also present, moderate inhibitors are obtained, with the 2′-methoxy-5′-chloro compound **7** being in the group of the 10 top inhibitors in the screening (Table 1).

Table 1. Turbidimetric Kinetic Assay [RA(%) and IC_{50} (μM)] Data for the Best 10 TTR Inhibitors (1−10)[a]

compound	RA (%)	IC_{50}
1 (200, IDIF)	92	4.1
2 (810)	77	5.7
3 (811)	92	5.5
4 (803)	87	5.2
5 (804)	99	3.3
6 (813)	92	5.0
7 (809)	87	6.1
8 (801)	95	5.6
9 (807)	87	9.5
10 (805)	81	8.9
tafamidis[b]	90	5.4
diflunisal[b] (201)	83	19

[a]See Table S8 in Supporting Information. IC_{50} values are the median of triplicate experiments with errors in the 7−10% experimental range. [b]Turbidimetric data on tafamidis and diflunisal are added for comparison purposes (in last two rows of the table).

Following the above observations, a subset of the 10 top best inhibitors from the screening, having very close IC_{50} activity values in the range of 3.7−10.0 μM, was selected. These compounds were individually resynthesized, purified by using conventional procedures and fully characterized, and rechecked for fibrillogenesis inhibition (Figure 3 and Table 1). Tafamidis was also included as a reference compound in these assays for comparison purposes.

The lead compound, iodiflunisal (IDIF, **1**) but not diflunisal was found among the selected compounds. Two pairs of twin iodinated/non iodinated analogues (**2** and **3**, and **4** and **5**) and four other unpaired noniodinated compounds are also in the group (**7**−**10**). The finding of these noniodinated highly active analogues among the best inhibitors supports the

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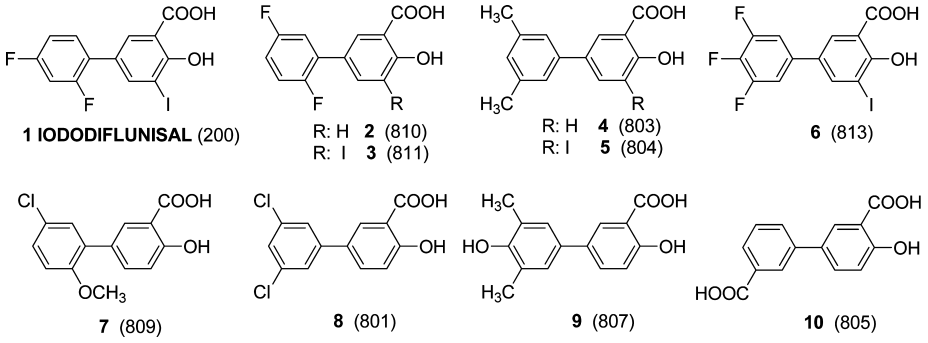

Figure 3. Selection of a subset of the best TTR inhibitors identified (in parentheses, compound numbering corresponds to the code in our database).

hypothesis that both iodination and the substitution pattern in the nonsalicylic ring are a crucial factor for activity.

These pure compounds were further studied for their binding potency to TTR by measuring displacement of radiolabeled thyroxine[21] (T_4) from TTR, which shows the affinity of the different compounds for TTR. Values of relative binding affinities thus obtained (data in Table S8 in Supporting Information) are plotted in Figure 4. Combined data on the 10

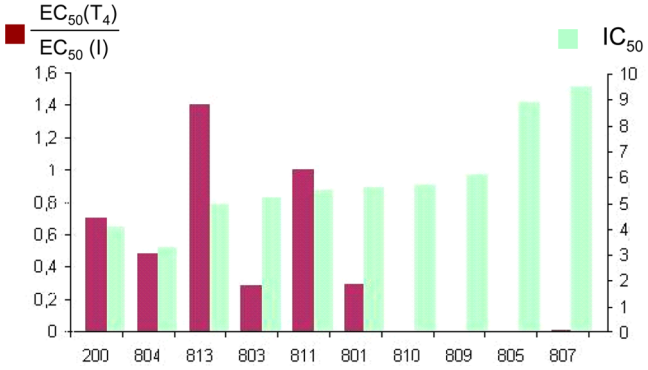

Figure 4. Values of relative binding affinities EC_{50} (T_4 displacement assay) and IC_{50} (turbidimetric kinetic assay) of selected TTR inhibitors (in pale blue, IC_{50}; in violet, EC_{50} rel).

best compounds confirmed that the best compound was the 3,5-dimethyl substituted analogue **5** (804) (3.3 μM), followed by iododiflunisal (**1**-200, IDIF, 4.1 μM), and the trifluorinated analogue **6** (813) (5.0 μM) (Figure 4 and Table S5 in the Supporting Information).

The iodinated diflunisal analogues show far higher affinity for TTR than the noniodinated. This is an indication that affinity seems to be strongly regulated by the iodine atom on the salicylic ring. In turn, this factor can be fine-tuned by the relative position and number of fluorine atoms in the difluorophenyl ring as shown by the trisubstituted fluorinated analogue **6** (813) which presents the highest binding potency.

In an attempt to initially explore in a more realistic system the interactions of these selected compounds with plasma proteins, we have qualitatively studied their relative affinity for TTR, TBG, and ALB using human plasma in the T_4 displacement test.[22] Interestingly, both iododiflunisal (**1**, IDIF) and the trifluorinated derivative **6** show the same pattern with good affinity for TTR, medium for TBG, and none to ALB. The other compounds either have less affinity for TTR or higher affinity for the other two proteins making them less selective for TTR (see Table S9 of the Supporting Information).

CONCLUSIONS

By using a two step solution-phase parallel synthesis, two related libraries of 77 diflunisal (5-aryl salicylic acids) and 77 iododiflunisal analogues (3-iodo-5-aryl salicylic acids) have been obtained. A new solid supported iodonium reagent prepared from Barluengás reagent (IPy_2BF_4) has been used for first time for polymer-assisted solution-phase parallel synthesis of a library of TTR amyloid inhibitors related to iododiflunisal. Thus, starting from 5-iodosalicylic acid and a set of 108 commercially available aryl boronic acids, the synthesis was conducted by combining an aqueous Suzuki cross-coupling methodology and an electrophilic aromatic substitution step affected by the immobilized iodonium version of Barluenga's reagent. A selected library of 77 noniodinated diflunisal analogues coming from the first reaction step and 77 iodinated diflunisal analogues coming from the second one indicated that the inhibition potency of diflunisal can be enhanced by substitution patterns on its difluorophenyl ring such as mono-, di-, or tri-substitutions, presenting polar (−OH and −COOH) and nonpolar groups which can be either halogen atoms (−F and −Cl) or simple aliphatic groups ($-CH_3$). Although no straightforward structure−activity relationships can be deduced from the present study, it is apparent that iodination does not generally improve the inhibition properties except for the compounds with 2′,4′- and 3′,5′- substitution in the nonsalicylic ring. In addition, by changing the relative position and the number of the fluorine atoms on this ring, we have discovered the 3′,4′,5′-trifluoro substituted iodinated diflunisal analogue **6** (813) which is the best inhibitor in this study because it combines high binding affinity as measured by competition for T_4 and low IC_{50} on the TTR fibrillogenesis inhibition test.

EXPERIMENTAL PROCEDURES

Compound Characterization. All compounds were obtained in milligram quantities. Details of the synthesis and characterization of each compounds is described in the Supporting Information (Compound Characterization). Structures of all reported compounds were confirmed by ^1H and ^{13}C NMR and their mass confirmed by HRMS. Purity was determined by HPLC analysis. All reported compounds had >95% purity. Selected spectra are included in the Supporting Information (Annex I).

General Procedure for the Synthesis of 5-Aryl Salicylic Acids from the Corresponding Aryl Boronic Acids and 5-Iodosalicylic Acid (Aqueous Suzuki-Miyaura Coupling). Under an argon atmosphere, 1.1 equiv of the arylboronic acid, 3 equiv of 2 M Na_2CO_3 and 4-iodosalicylic acid (1 equiv) are

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added to 10 mL of deaerated H_2O. The catalyst $Pd(OAc)_2$ (1% mol) is added, and the reaction is stirred at rt for a period of 2−4 h. The reaction is monitored by TLC and/or HPLC. The crude reaction is dissolved in 100−150 mL of H_2O or in a H_2O/dioxane (2:1) mixture, filtered through Celite and acidified to pH = 2 with a 1 N HCl solution until a white precipitate is formed. The crude is separated either by centrifugation or by extraction with ethyl acetate and dried in a desiccator under P_2O_5. The crude is purified by silica gel column chromatography.

General Procedure for Conversion of 5-Aryl Salicylic Acids to the Corresponding 5-Aryl-3-Iodosalicylic Acids with (a) IPy_2BF_4 or (b) with the Polymer-Supported Iodonium Reagent (PV-Py-I-Py). *a. With IPy_2BF_4.* To a solution of the 5-aryl salicylic acid (1 equiv) in 4−10 mL of CH_2Cl_2, a solution of the iodonium reagent IPy_2BF_4 (1.5 equiv) in 2−4 mL of the same solvent was added. The mixture is stirred at rt for 1.5−2 h and the reaction monitored by HPLC. After completion, water is added to the crude and extracted, and the organic phases are washed with sodium thiosulfate and brine. The organic phase is evaporated under reduced pressure, and the crude is dried under reduced pressure. In some cases the crude is purified by silica gel column chromatography.

b. With the Polymer-Supported Iodonium Reagent (PV-Py-I-Py). To a solution of 5-aryl salicylic acids (1 mmol) in methanol (10 mL), polymer-supported iodonium reagent (3 mmol) was added and the mixture was allowed to stir at 50 °C. The progress of the reaction was followed by HPLC. When the reaction was completed, the resin was filtered and washed successively with methanol. The solvent was evaporated, and the pure product was obtained in moderate to high yields (60−80% yield).

Representative Examples: Compound (8 or 801) and (4 or 803). *3′,5′-Dichloro-4-hydroxy-[1,1′-biphenyl]-3-carboxylic Acid (**8** or 801).* From 3′,5′-dichlorophenylboronic acid (0.209 g, 1.1 mmol), Na_2CO_3 (0.318 g, 3 mmol), 5-iodosalicylic (0.264 g, 1 mmol), and $Pd(OAc)_2$ (0.002 g, 0.01 mmol) yielded 0.238 g (84%). HPLC (GEN-1) RT: 13.46 min. ^{1}H NMR (500 MHz; DMSO-d6) δ (ppm): 8.05 (d, J = 2.5 Hz, 1H), 7.86 (dd, J = 2.5, 9.0 Hz, 1H), 7.65 (d, J = 1.5 Hz, 2H), 7.51 (t, J = 2.0 Hz, 1H), 7.04 (d, J = 9.0 Hz, 1H). ^{13}C NMR (125.7 MHz; DMSO-d6) δ (ppm): 171.4, 161.3, 142.6, 134.6, 133.9, 128.5, 128.2, 126.3, 124.8, 117.9, 113.7. MS (ESI$^-$) m/z 281 (M − H)$^-$. HRMS (ESI$^-$): calcd for $[C_{13}H_8Cl_2O_3 - H]^-$, 280.9772; found, 280.9771.

*4-Hydroxy-3′,5′-dimethyl-[1,1′-biphenyl]-3-carboxylic Acid (**4** or 803).* From 3,5-dimethylphenylboronic acid (0.165 g, 1.1 mmol), Na_2CO_3 (0.318, 3 mmol), 5-iodosalicylic acid (0.264 g, 1 mmol), and $Pd(OAc)_2$ (0.002 g, 0.01 mmol) yielded 0.178 g (73%). HPLC (GEN-1) RT: 11.16 min.^{1}H NMR (500 MHz; CD_3COCD_3) δ (ppm): 8.12 (d, J = 2.5 Hz, 1H), 7.81 (dd, J = 2.5, 8.5 Hz, 1H), 7.23 (m, 2H), 7.03 (d, J = 8.5 Hz, 1H), 6.98 (m, 1H), 2.33 (s, 6H). ^{13}C NMR (125.7 MHz; CD_3COCD_3) δ (ppm): 172.6, 162.3, 140.5, 139.1, 135.3, 133.3, 129.5, 129.1, 125.1, 118.6, 113.3, 21.4. HRMS (ESI$^-$): calcd for $[C_{15}H_{14}O_3 - H]^-$, 241.0865; found, 241.0853.

Kinetic Turbidimetric Assay. Inhibition of fibrillogenesis was determined by the kinetic turbidimetric assay previously reported.[20] Briefly, in seven different wells of a 96-well microplate, 20 μL of a 4 mg/mL TTRY78F solution in 20 mM potassium phosphate buffer, 100 mM KCl, and 1 mM EDTA at pH 7.6 was mixed with 80 μL of a solution of inhibitor prepared by mixing different volumes of a stock solution of the compound in H_2O/DMSO (1:1) to give a range of final compound concentrations of 0−40 μM and DMSO content adjusted to a final 5% (v/v), where all ligands tested are soluble. After 30 min incubation at 37 °C with 15 s shaking every minute, 100 μL of 400 mM KAcO, 100 mM KCl, and 1 mM EDTA buffer at pH 4.2 were added to each well. The final mixture, containing 0.4 mg/mL TTR, 0−40 μM ligand, and 5% DMSO, was incubated at 37 °C with 15 s shaking every minute. Absorbance at 340 nm was monitored for 1.5 h at 1 min intervals. Initial rates of protein aggregation (v_0) were obtained from the linear plot absorbance vs time. The dependence of v_0 on inhibitor concentration is defined as

$$v_0 = A + B \exp^{-C[I]} \quad (1)$$

where v_0 is the initial rate of fibril formation (in absorbance units per hour, AU h^{-1}) and [I] the concentration of the inhibitor (μM).

From the adjustable parameters, the IC_{50} (inhibitor concentration at which the initial rate of protein aggregation is half than that in absence of inhibitor) and RA (%) (percentage reduction of amyloidosis at high inhibitor concentration) were calculated.

Competition with T_4 and Binding Selectivity Assays. Binding to TTR is not the exclusive factor in determining the therapeutic potential of new fibrillogenesis inhibitory compounds. Binding specificity to plasma proteins can be a limiting factor for biodistribution, metabolism, activity, and toxicity profiles of any potential drug. This is especially crucial in this case because very strong plasma protein competitors of TTR include thyroxine-binding globulin (TBG), which has an order of magnitude higher affinity for thyroxine, and albumin (ALB) which is at concentrations of 2 orders of magnitude higher than TTR in plasma.

Binding Competition between Compounds and T_4. To evaluate the relative binding affinity of the compounds for TTR, an already described procedure was followed. Wild type recombinant TTR was incubated with trace amounts of ^{125}I-T_4 in the presence of increasing amounts of test compounds. Protein bound ^{125}I-T_4 was separated from the media by gel filtration and measured by scintillation. Competition curves allowed calculating the relative T4 displacement potencies defined as EC50 of T_4/EC50 of the test compound for each inhibitor.

Binding Selectivity for Plasma T_4 Binding Proteins. Human plasma was incubated with labeled T_4 (^{125}I-T_4) in the presence of each inhibitor. After separation of plasma proteins by native polyacrylamide gel electrophoresis (PAGE), qualitative T_4 displacement binding from TBG, ALB, and TTR (main plasma T_4 binding proteins) was measured either by autoradiography or by phosphorimaging of the dried gel.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis and characterization of the combinatorial libraries of diflunisal and iododiflunisal analogues, fibrillogenesis inhibition parameters of compounds, data on TTR binding assays, and selectivity assays of the best compounds; polymer-supported iodonium reagent preparation and characterization; and selected spectra (HPLC, ^{1}H NMR, ^{13}C NMR, HRMS) of the best target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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DEDICATION

This paper is dedicated to Prof. Dr. José Barluenga on his 74th birthday.

ABBREVIATIONS

ALB, albumin; AcOH, acetic acid; DMSO, dimethyl sulfoxide; EDTA, ethylendiaminetetraacetic acid; EtOAc, ethyl acetate; HRMS, high-resolution mass spectrometry; HPLC, high-performance liquid chromatography; IDIF, iododiflunisal; IPy_2BF_4, *bis*(pyridine)iodonium(I) tetrafluoroborate; KAcO, potassium acetate; MS (ESI^-), mass spectrometry (electrospray ionization, negative mode); PV-Py, poly(4-vinylpyridine) beads; PV-Py-I-Py, polymer-supported iodonium reagent; R_f, retention factor; RT, retention time; T_4, thyroxine; TTR, transthyretin; TBG, thyroxine-binding globulin; TFA, trifluoroacetic acid; TLC, thin layer chromatography; TTR, transthyretin; Y78F, transthyretin mutant with Phe replacing Tyr at position 78

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