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Design, Synthesis, and Structure−Activity Relationship of *N*-Arylnaphthylamine Derivatives as Amyloid Aggregation Inhibitors

Roberto Di Santo,*[,†] Roberta Costi,[†] Giuliana Cuzzucoli Crucitti,[†] Luca Pescatori,[†] Federica Rosi,[†] Luigi Scipione,[†] Diana Celona,[‡] Mario Vertechy,[‡] Orlando Ghirardi,[‡] Paola Piovesan,[‡] Mauro Marzi,[‡] Silvio Caccia,[§] Giovanna Guiso,[§] Fabrizio Giorgi,[‡] and Patrizia Minetti*[,‡]

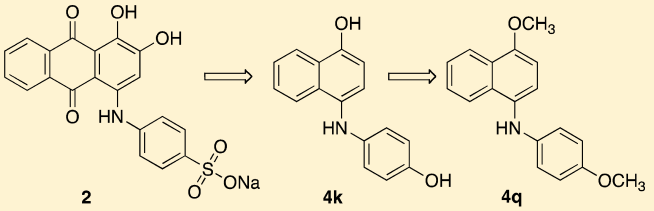
[†]Dipartimento di Chimica e Tecnologie del Farmaco, Istituto Pasteur—Fondazione Cenci Bolognetti, "Sapienza" Università di Roma, P.le A. Moro 5, I-00185 Roma, Italy

[‡]Sigma-Tau S.p.A., Via Pontina, Km 30.400, I-00040 Pomezia, Italy

[§]Dipartimento di Neuroscienze, Istituto di Ricerche Farmacologiche "Mario Negri", Via La Masa, 19, I-20156 Milano, Italy

Supporting Information

ABSTRACT: Dyes like CR are able to inhibit the aggregation of Aβ fibrils. Thus, a screening of a series of dyes including ABBB (**1**) was performed. Its main component **2** tested in an in vitro assay (i.e., ThT assay) showed good potency at inhibiting fibrils association. Congeners **4**−**9** have been designed and synthesized as inhibitors of Aβ aggregation. A number of these newly synthesized compounds have been found to be active in the ThT assay with IC_{50} of 1−57.4 μM. The most potent compound of this series, **4k**, showed micromolar activity in this test. Another potent derivative **4q** (IC_{50} = 5.6 μM) rapidly crossed the blood−brain barrier, achieving whole brain concentrations higher than in plasma. So **4q** could be developed to find novel potent antiaggregating βA agents useful in Alzheimer disease as well as other neurological diseases characterized by deposits of amyloid aggregates.



INTRODUCTION

Alzheimer's disease (AD) is a degenerative brain disorder and a major cause of dementia for which no cure is known. Currently, more than 35 million people worldwide are believed to suffer from this disease, and with the aging of the population this number is expected to increase by a factor of 3−4 over the next 40 years.[1]

The presence of extracellular β-amyloid (Aβ) peptide fibrils deposits and the presence of intracellular neurofibrillary tangles (NFT) made by abnormally hyperphosphorylated protein tau are among the clearest signs of AD. These two events, which involve mainly the medial temporal lobe structures, in particular the hippocampus and cortical areas of the brain, are necessary conditions for the onset of the disease, even if the final pathological picture of the disease involves the whole central nervous system.[2,3] These microscopic aggregates are still well beyond the resolution of conventional neuroimaging techniques (positron emission tomography, computerized tomography, magnetic resonance imaging) used for the evaluation of patients with AD. Selective tau imaging for in vivo NFT quantification is still in the early stages of development.[4] Since Aβ is at the center of AD pathogenesis and given that several pharmacological agents aimed at reducing Aβ levels in the brain are being developed and tested, many efforts have focused on generating radiotracers or agents that allow Aβ imaging in vivo.[5−7]

The main constituents of Aβ fibrils, $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$, normally present as soluble species in extracellular fluids, twisted around each other, resulting in filamentous structures with a diameter of 6−10 nm. Amyloid fibrils have been defined as thermodynamically stable, structurally organized, and highly insoluble, having affinity for the Congo Red (CR) stain[8] and filamentous protein aggregates being composed of repeating units of β-sheets aligned perpendicular to the fiber axis, with potentially parallel and antiparallel organization.[9] In AD, the amyloid fibers accumulate outside the cell in the extracellular spaces of the brain and in the tunica media of the cortical and meningeal arterioles, producing three different macroscopic changes: senile plaques and diffuse plaques which can be differentiated by the presence or absence of modifications in the neuronal processes around the central amyloid deposit, and amyloid angiopathy, which represents an infiltration of amyloid fibers between the smooth muscle fibers and the internal elastic lamina of the arterial wall.[10] The clinical manifestations of this progressive and irreversible dementia, in which are implicated the $A\beta_{(1-42)}$ aggregated fibrils, are the progressive loss of memory and cognitive functions.[11] In general, the damage caused by Aβ aggregation may be summarized by the following: (i) changes in amyloidogenesis; (ii) increase in the vulnerability

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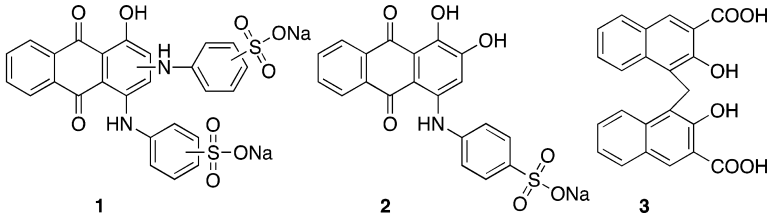

Figure 1. **1**, **2** (main component of a commercial mixture of **1**), and **3** (pamoic acid).

of neurons to excitotoxicity; (iii) increase in the vulnerability of the neurons to hypoglycaemic damage; (iv) changes in the homeostasis of calcium; (v) increase in damage by oxidation; (vi) activation of inflammatory mechanisms; (vii) activation of the microglia; (viii) induction of lysosomial proteases; (ix) changes in the phosphorylation of the protein tau; (x) induction of apoptosis; (xi) damage to membranes.[12]

Since the toxicity and neurodegeneration in AD are attributed to soluble intermediate oligomers and to a lesser extent to amyloid fibrils,[13] agents that prevent or reverse the oligomerization and/or fibrillization of Aβ may have potential therapeutic application in the treatment of AD.[14] From a purely theoretical point of view, the reduction of the damage caused by Aβ can be addressed through different therapeutic approaches: (i) reducing the production of Aβ using inhibitors of the secretases to change the metabolism of the APP (increasing the α or reducing the β and γ secretases); (ii) preventing or blocking the aggregation of the Aβ; (iii) increasing the clearance of the Aβ; (iv) blocking the neurotoxic effects of Aβ restoring calcium homeostasis; (v) preventing the toxicity due to the free radicals; (vi) preventing excitotoxicity; (vii) reducing the damage caused by the inflammatory response; (viii) correcting the imbalance between zinc and copper; (ix) inhibiting neuronal apoptosis.[15−17] Efforts at searching for inhibitors of Aβ aggregation have demonstrated that small molecules, containing single or multiple (hetero)-aromatic rings often bearing hydroxyl group, can inhibit protein−protein interaction.[18] Several molecular dynamics studies suggest that the driving forces governing the Aβ assembly might include aromatic packing, hydrophobic forces, and electrostatic interactions.[19−21] The hydrophobic interaction between Aβ side chain leading to Aβ fibrillization could be blocked by small molecules competing with such an association, ultimately leading to inhibition of fibril formation.[22] These interactions could be reinforced by hydrogen bond formation between hydroxyl or methoxy groups on aromatic rings of the inhibitor and hydrogen bond acceptor groups of Aβ.

To date, no specific therapy exists to prevent, slow down, or arrest the amyloidogenic processes at the root of AD. Indeed the treatments currently used for this disease are exclusively symptomatic, and even if they act on various aspects, they fundamentally only interfere with the neurotransmitter mechanisms, which govern learning and memory. The substances mostly used include the reversible inhibitors of acetylcholinesterase, such as tacrine, donepezil, and rivastigmine.[23]

Our initial attempts at generating small molecule inhibitors of Aβ aggregation were based on the knowledge that CR showed some affinity for amyloid protein fibrils. Thus, we decided to screen a few dyes as potential inhibitors of the Aβ aggregation, and among them we found that alizarin blue-black B (ABBB) (**1**) (Figure 1) (Sigma-Aldrich mordant black [1324-21-6]) showed interesting potency in inhibiting the fibrils association in the ThT assay.[24,25]

However, **1** as sold by Aldrich was well-known to be a mixture of anthraquinone derivatives that necessitated purification for elucidation of the nature of the various components. Within the commercial mixture of **1** we isolated and identified the major component (~40%) as the 3,4-dihydroxy-1-[1-(4-sulfophenylamino)]anthracene-9,10-dione (**2**) and tested it for its ability to inhibit the Aβ aggregation in the ThT assay. Compound **2** was an efficient inhibitor showing IC_{50} = 1.1 μM. In parallel, during research performed at our company on negatively charged compounds as inhibitors of Aβ aggregation, we discovered that pamoic acid (**3**) (Figure 1) demonstrated some inhibiting properties with regard to Aβ aggregation in in vitro assays, showing IC_{50} = 38.2 μM.

The above data stimulated us to design new compounds structurally related to **2** and **3** as Aβ aggregation inhibitors. In particular, *N*-arylnaphthylamine derivatives **4**, in which the anthraquinone moiety of **2** was replaced by naphthalene ring typical of compound **3**, were designed (Figure 2). This replacement

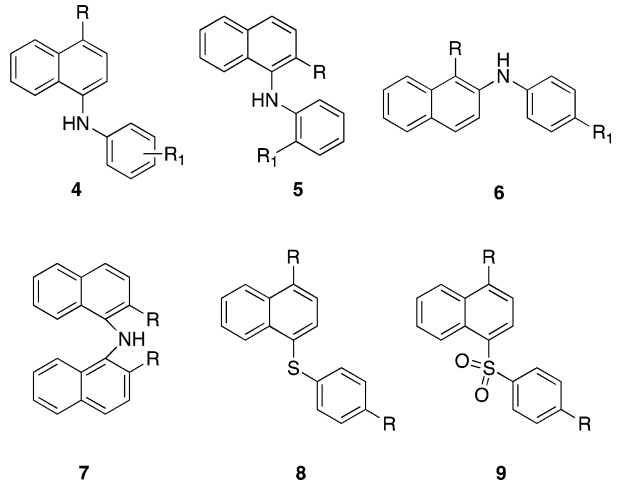

Figure 2. *N*-Arylnaphthylamines (**4**−**7**) and thio analogues **8** and **9** designed as Aβ aggregation inhibitors (for substituents R and R_1, see Table 1).

was motivated by the fact that 9,10-anthraquinone derivatives, such as antitumor agent adriamycin, are known to generate in vivo toxic metabolites that are particularly noxious for heart tissues.[26]

Some analogues of **4** such as compounds **5**−**8** were further designed to better define the structure−activity relationship (SAR) within this class of inhibitors (Figure 2). As a first step in these preliminary SAR studies, N and S linkers between the two aromatic rings were chosen essentially because of easier synthetic feasibility.

The rationale to design *N*-arylnaphthylamines as inhibitors of Aβ aggregation has also been strengthened by reports on *N*-phenylanthranilic derivatives, represented by compounds **10**−**12**, which have been recently described as Aβ aggregation inhibitors (Figure 3).[27]

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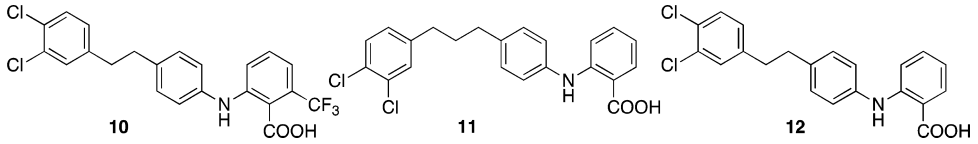

Figure 3. *N*-Phenylanthranilates reported in literature that inhibit Aβ aggregation.

Thus, in the present study we describe a series of novel *N*-arylnaphthylamines (**4**–**7**) and thio analogues **8** and **9** (Figure 2 and Table 1) as potential inhibitors of Aβ aggregation in in vitro assays, using the ThT.[24]

As typical substituents R and R_1 (see Table 1), hydroxyls and carboxylic acids, including salicylic moieties, were chosen to mimic substituents that characterize compounds **1**–**3** and **10**–**12**. Some replacement groups were also attempted to better define the SAR within this class of inhibitors. Furthermore, since the blood–brain barrier crossing always represents one of the main problems for the compounds aimed at acting on the central nervous system, we also performed in vivo preliminary studies on mice and assessed the ability of the most interesting inhibitor to cross the blood–brain barrier.

RESULTS AND DISCUSSION

Chemistry. *Analysis.* Mixture **1** as furnished by Aldrich (mordant black 1324-21-6), was analyzed by LC–MS using a RP18 column and ESI-MS detector. Various peaks were found, and the main one was quantified to represent around 40% of the mixture based on UV detection. After separation by semipreparative HPLC by means of a 1 cm RP18 column, this component was unequivocally identified to be 4a,9a-dihydro-3,4-dihydroxy-1-[1-(4-sulfophenylamino)]anthracene-9,10-dione (**2**) by NMR and MS analyses.

Synthesis. Syntheses of compounds **4**–**7** have been made through N-arylation of a substituted naphthylamine with the appropriate aryl halide by means of the Buchwald palladium-catalyzed amination reaction using a mixture of palladium acetate ($Pd(OAc)_2$) and racemic 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) as catalysts, in the presence of Cs_2CO_3. Thus, *N*-arylnaphthylamines **4b**,**m**,**o**–**r**,**t**, **5c**, and **6d**,**f**,**g** have been synthesized in the cited conditions (Scheme 1). The above-described reaction performed on 4-nitro-1-naphthalenamine with 4-bromoanisole as electrophile agent gave very poor yield (<5%) because of the low reactivity of the deactivated naphthalenamine. Thus, we decided to invert the reactivity of the reagents using 4-iodo-1-nitronaphthalene as electrophile and 4-methoxyaniline as nucleophile agents, obtaining nitro derivative **4e** in good yields (Scheme 1). This approach was also used for compounds **4c**,**d**,**f** using the appropriate bromonaphthalene derivative and the properly substituted aniline (Scheme 1).

Deprotection of methoxy groups of **4m**,**q**, and **6d** was performed with BBr_3 at −45 °C to furnish **4g**,**k** and **6a**, respectively (Scheme 2).

The same reaction was used to deprotect methoxy and/or carboxymethyl groups of **4o**,**t**, **5c**, and **6f** to furnish **4i**,**j**,**l**, **5a**,**b**, and **6b**,**c**, respectively (Schemes 2 and 3).

Partial deprotection was obtained by performing the reaction at a controlled temperature. In fact, the methoxy group of compound **4o** was selectively deprotected at −45 °C to give the corresponding phenol **4j**. Conversely, when the temperature of the reaction was increased to room temperature with a longer reaction time (15 h), the carboxymethyl group was deprotected as well to obtain **4i** (Scheme 3). However, a more convenient route to obtain both hydroxy ester and hydroxy acid derivatives was found by performing the reaction at room temperature with a short reaction time (∼30 min). In such a way, deprotection of **5c** and **6f** gave a mixture of **5a**, **5b** and **6b**, **6c**, respectively, which were separated by chromatography (Schemes 2 and 3). Further, the deprotection of **4t** at room temperature for 15 h gave only the salicylic derivative **4l** (Scheme 2).

Carboxylic acids **4a**,**n**,**s** and **6e** were obtained by basic hydrolysis of the corresponding esters **4b**,**o**,**t** and **6f** (Scheme 3).

Finally, the carbinol **4h** was obtained starting from the corresponding methyl ester **4j** by reduction with $LiAlH_4$ (Scheme 2). Unfortunately, derivative **4h** was unstable and degraded rapidly after workup of the reaction.

Bis-naphthylamines **7** were obtained through a route similar to that described for compounds **4** and **6**. In particular, N-arylation was performed starting from 1-bromo-2-methoxynaphthalene and 2-methoxy-1-naphthalenamine in the presence of $Pd(OAc)_2$, BINAP, and Cs_2CO_3. The bis-naphthylamine **7b** that formed was then deprotected using BBr_3 to afford **7a**, which proved to be unstable (Scheme 4).

The synthesis of thio derivatives **8** and **9** is reported in Scheme 5. The arylation step was obtained by palladium catalyzed reaction of 4-methoxythiophenol, using 1-iodo-4-methoxynaphthalene as arylating agent in the presence of tris(dibenzylideneacetone)dipalladium (Pd_2dba_3) and bis(2-diphenylphosphinophenyl) ether (DPEphos) as catalysts and *t*-BuOK (Scheme 5). This reaction afforded derivative **8b**, which was deprotected in the presence of BBr_3 to furnish the phenol **8a** or oxidized to afford sulfone **9b**, which was in turn deprotected to give the phenol **9a**.

Evaluation of Biological Activities. In Vitro Assays. The antiaggregating activity of compounds **4**–**9** on the peptide A$\beta_{(1-42)}$ has been determined via the binding of the ThT according to the following procedure and is reported in more detail in the Experimental Section. Test compounds were added in the wells containing both the aggregate and the nonaggregate A$\beta_{(1-42)}$, incubated at 37 °C under agitation for 24 h. ThT was added to each well, and the fluorescence was measured. The assay was performed in triplicate in 96-well plates, and the data obtained were expressed as the dose reducing the aggregate formation by 50% (IC_{50}) (Table 1).

Among the 36 newly synthesized arylamines **4**–**9**, 29 compounds were tested as reported above. The remaining compounds were not submitted to the ThT assay, since two compounds (**4h** and **7a**) were unstable and five derivatives (**4m**,**o**, **6c**, **8b**, **9b**) had poor solubility in the assay conditions. Derivatives **4a**–**d**,**n**,**p**,**r**–**t**, **5b**,**c**, **6e**–**g**, and **9a** were found to be inactive when tested at the highest concentration allowed by their solubility. Conversely, the remaining 14 compounds showed IC_{50} ranging from 1 to 57.4 μM. In particular, nine compounds (**4e**–**g**,**j**,**k**,**q**, **6a**,**b**, and **7b**) were active at micromolar concentrations (IC_{50} ranging from 1 to 5.7 μM), with the dihydroxy derivative **4k** being the most potent one within this series, with IC_{50} = 1 μM being comparable to the ones of alizarin derivative **2** and antranilates **11** and **12** and 38 times more potent than pamoate **3**.

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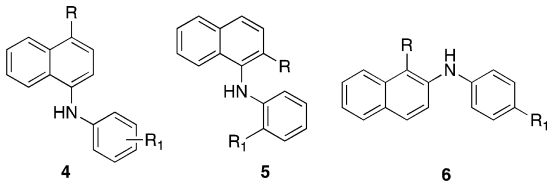
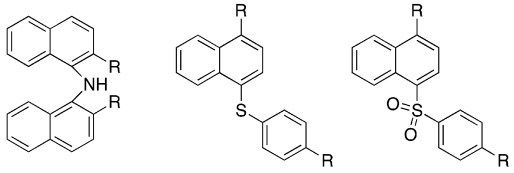
Table 1. Antiaggregating Activity of 4a–t, 5a–c, 6a–g, 7a,b, 8a,b, 9a,b, and Reference Compounds 2, 3, and 10–12 on the Peptide Aβ





compd	R	R_1	IC_{50} (μM)[a]
4a	H	4-COOH	>100
4b	H	4-$COOCH_3$	>20
4c	F	4-F	>30
4d	F	4-SCH_3	>5
4e	NO_2	4-OCH_3	2.7
4f	$N(CH_3)_2$	4-SCH_3	2.2
4g	OH	H	5.4
4h	OH	4-CH_2OH	ND
4i	OH	4-COOH	57.4
4j	OH	4-$COOCH_3$	5.1
4k	OH	4-OH	1
4l	OH	3-COOH-4-OH	46.1
4m	OCH_3	H	ND
4n	OCH_3	4-COOH	>60
4o	OCH_3	4-$COOCH_3$	ND
4p	OCH_3	2-OCH_3	>5
4q	OCH_3	4-OCH_3	5.6
4r	OCH_3	2-OCH_3-5-I	>5
4s	OCH_3	3-COOH-4-OCH_3	>80
4t	OCH_3	3-$COOCH_3$-4-OCH_3	ND
5a	OH	COOH	14.1
5b	OH	$COOCH_3$	>20
5c	OCH_3	$COOCH_3$	>5
6a	OH	H	2.4
6b	OH	COOH	2.6
6c	OH	$COOCH_3$	ND
6d	OCH_3	H	33.6
6e	OCH_3	COOH	>20
6f	OCH_3	$COOCH_3$	>20
6g	OCH_3	I	>5
7a	OH	OH	ND
7b	OCH_3	OCH_3	5.7
8a	OH	OH	10.9
8b	OCH_3	OCH_3	ND
9a	OH	OH	>100
9b	OCH_3	OCH_3	ND
2			1.1
3			38.2
10			0.1
11			1
12			2

[a]Inhibitory concentration, 50% (μM), determined from dose–response curves. Experiments were performed in triplicate.

Removal of the phenolic OH group of **4k** or its replacement with $COOCH_3$ led to arylamines **4g** and **4j**, which were about 5-fold less potent than the parent compound (**4g**, IC_{50} = 5.4 μM; **4j**, IC_{50} = 5.1 μM). Conversely, its replacement with a COOH (**4i**) or transformation in a salycilic derivative (**4l**) gave a more substantial drop in activity (**4i**, IC_{50} = 57.4 μM; **4l**, IC_{50} = 46.1 μM).

Replacement of both hydroxyls of **4k** with OCH_3 groups led to naphthylarylamine **4q**, which was about 5-fold less potent than the parent compound (IC_{50} = 5.6 μM). However, generally the methylation of OH gave inactive compounds or compounds with low activity (compare **4g,i–l** with **4m–t**).

Compounds with no hydroxyl groups such as **4a–d** were inactive. In particular, replacement of the OH of **4k** with a fluorine atom led to fluoroderivative **4c** that showed IC_{50} > 30 μM.

The methylthiofluoro derivative **4d** was inactive as well, while the corresponding compound **4f**, in which the fluorine atom was replaced by a dimethylamino group, showed very good potency (IC_{50} = 2.2 μM).

Although the presence of OH groups seems to be relevant for activity, it is not vital because its replacement with groups such as COOH, $COOCH_3$, OCH_3, NO_2, $N(CH_3)_2$, SCH_3 led to compounds active in the micromolar range (compare **4k** with **4e–g,j,q**, and **6a,b**).

Shift of substituents of **4i** from the 4- to 2-position of the aromatic rings led to **5a**, which was 4-fold more potent than **4i** (**4i**, IC_{50} = 57.4 μM; **5a**, IC_{50} = 14.1 μM). However, introduction of less polar groups within the series of compounds **5** gave results similar to those obtained for series **4**, giving inactive derivatives **5b,c**.

Compound **6a**, the isomer of **4g** in which the 4-hydroxyphenylamino moiety is transposed from the 4- to 2-position of the naphthalene ring, was about 2 times more potent than the parent compound (**4g**, IC_{50} = 5.4 μM; **6a**, IC_{50} = 2.4 μM). An even stronger effect was obtained for the corresponding acid derivatives **4i** and **6b**. In fact, compound **6b** showed IC_{50} = 2.6 μM and was 22 times more active than **4i** (IC_{50} = 57.4 μM). Interestingly, introduction of a carboxylic group in the 4-position of the phenyl ring of **6a** gave **6b**, which was equipotent to the parent compound (**6a**, IC_{50} = 2.4 μM; **6b**, IC_{50} = 2.6 μM). Conversely, a similar modification in the **4** series gave **4i** that was about 10-fold less potent than **4g** (**4i**, IC_{50} = 57.4 μM; **4g**, IC_{50} = 5.4 μM). As confirmation of a general rule, phenol derivatives of the **6** series were more potent if compared to the less polar counterparts (see **6a,b** and **6d–g**).

Replacement of the phenyl ring of **4q** with a naphthalene moiety gave the equipotent bis-naphthalenylamino derivative **7b** (**4q**, IC_{50} = 5.6 μM; **7b**, IC_{50} = 5.7 μM). No comparison was possible between the corresponding dihydroxyderivatives **4k** and **7a**, since the bis-naphtholamine derivative **7a** was unstable.

Isosteric substitution of the linker of **4k** with replacement of NH with a sulfur atom gave compound **8a**, which was 11 times less active than the corresponding amine (**4k**, IC_{50} = 1 μM; **8a**, IC_{50} = 10.9 μM). The oxidation of the linker of **8a** led to the inactive sulphone **9a**. Finally, both the dimethoxy derivatives **8b** and **9b** were too scarcely soluble to be tested.

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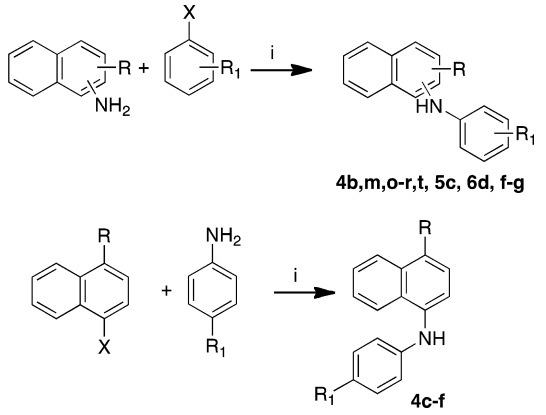
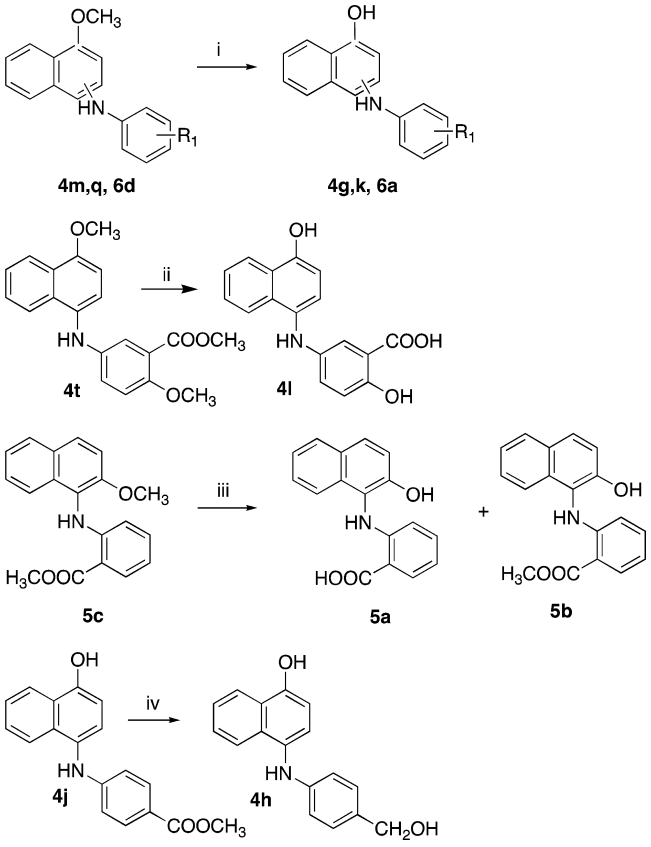
Scheme 1[a]



[a]Reagents and conditions: (i) $Pd(OAc)_2$, (±)-BINAP, Cs_2CO_3, 80 °C. For substituents R and R_1, see Table 1.

Scheme 2[a]



[a]Reagents and conditions: (i) BBr_3, CH_2Cl_2, −45 °C; (ii) BBr_3, CH_2Cl_2, rt, 15 h; (iii) BBr_3, CH_2Cl_2, rt, 30 min; (iv) $LiAlH_4$, THF, rt. For substituents R_1, see Table 1.

Brain-to-Plasma Distribution Studies. The aim of these animal model studies was to evaluate the distribution between brain and plasma of *N*-arylnaphthylamines. Compound **4q** was selected as a representative derivative of the series because of a good balance between activity and lipophilicity as well as acceptable chemical stability. However, **4k**, which was the most potent compound within this series and proved to be 5−6 times more potent than **4q** in in vitro assays, showed oxidative degradation.

Figure 4 shows the plasma and brain concentration−time curves of compound **4q** after subcutaneous dosing in mice. Subcutaneous absorption was relatively rapid as indicated by the t_{max}, which was 60 min after a 25 mg/kg dose (dissolved in ethanol, PEG-400, saline, 10:40:50 v/v/v). The C_{max} was 164 ng/mL (n = 3) and AUC_t was 850 ng/(mL·h). Plasma concentrations of **4q** fell slowly thereafter, about 80% of the plasma C_{max} remaining after 6 h. However, in these preliminary studies, plasma sampling was not sufficiently long for a proper determination of half-life and of other conventional pharmacokinetic parameters.

From the plasma concentration−time curve, the compound achieved brain C_{max} at 60 min. However, brain C_{max} (410 ng/mL; n = 3) was 2.5 times that in plasma, indicating concentration of this *N*-arylnaphthylamine derivative in brain tissue.

After the peak, mean brain concentrations remained consistently 1.7- to 2.5-fold the plasma concentrations within 6 h of dosing, yielding a mean brain-to-plasma AUC_t ratio of about 2. This led us to conclude that **4q**, one of the most potent derivatives of the present series, rapidly entered the mouse central nervous system and equilibrated between plasma and brain.

Compound **4q** was well tolerated, since no discomfort or side effects were observed.

CONCLUSIONS

On the basis of the knowledge that a few dyes like CR were able to inhibit the aggregation of Aβ fibrils, we perfomed a screening of a series of dyes. Mixture **1** was found to be active and its main component, anthraquinone derivative **2** which was isolated and unequivocally identified, was tested for its antiaggregating properties in in vitro assay. Interestingly, compound **2** showed good potency in inhibiting the fibrils association in the ThT assay (IC_{50} = 1 μM). So a series of *N*-arylnaphthylamine derivatives (**4**−**7**) and thio analogues **8** and **9** were designed as Aβ aggregation inhibitors. A number of the newly synthesized compounds have been found to be active with IC_{50} ranging from to 1 to 57.4 μM. The most potent compounds showed IC_{50} in the low micromolar range, comparable to that of hit compound **2**. Furthermore, these novel *N*-arylnaphthylamine derivatives are potentially less toxic than the anthraquinone derivative **2** and one of the more interesting derivatives (**4q**) rapidly crossed the blood−brain barrier and diffused within the central nervous system, achieving concentrations about twice those in plasma, looking at the whole brain exposure. This brain-to-plasma ratio was similar to that of donezepil (1.74−2.24), slightly lower than that of rivastagmine and tacrine in rats (about 3 and 5, respectively),[28−30] but it remains to be clarified whether such observations reflect the true extent of brain distribution, since the plasma protein binding of **4q** was not evaluated in this study. So **4q** represents a very interesting compound that could be developed with the aim to find new potent antiaggregating Aβ agents useful for the treatment of Alzheimer disease as well as other neurological diseases characterized by deposits of amyloid aggregates.

EXPERIMENTAL SECTION

Chemistry. General. Melting points were determined on a Bibby Stuart Scientific SMP1 melting point apparatus and are uncorrected. The purity of the compounds was always >95% as determined by high pressure liquid chromatography (HPLC). HPLC analyses were carried

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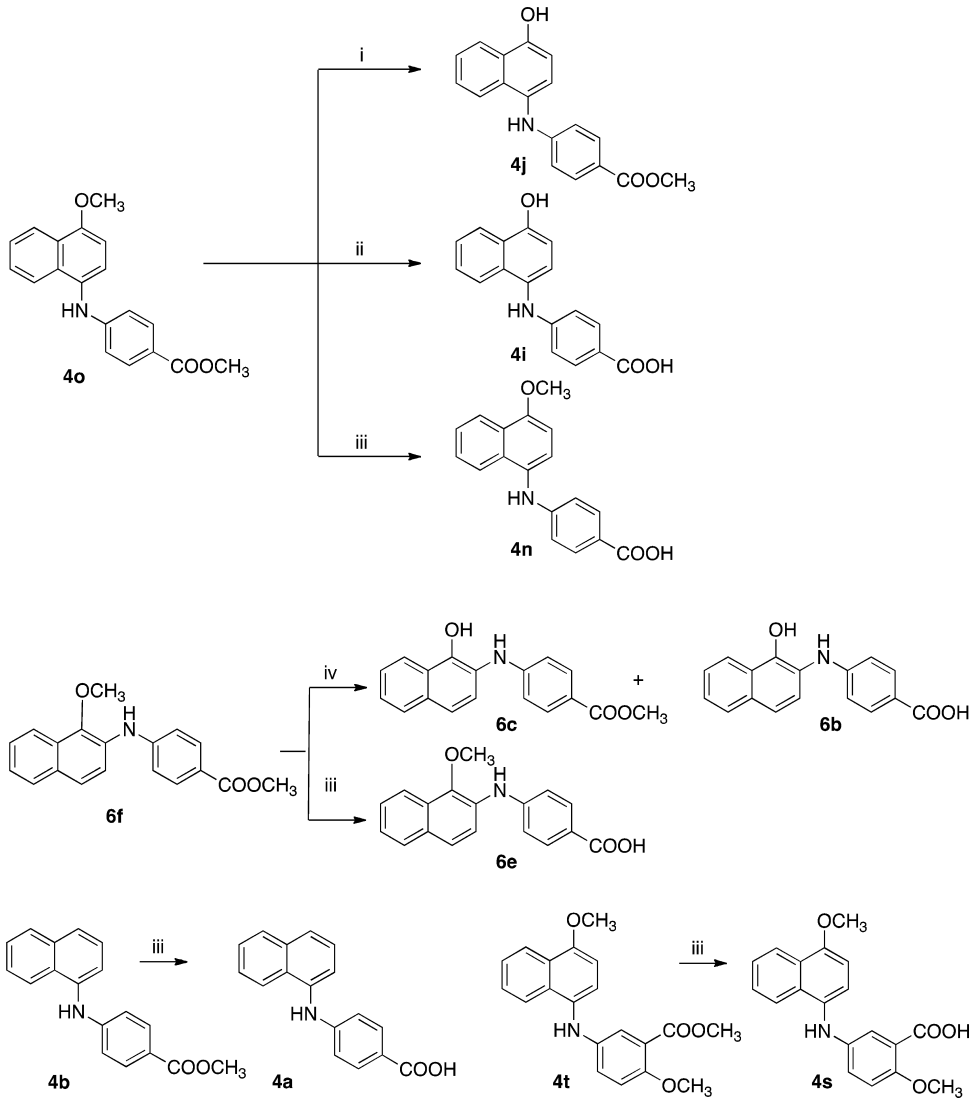
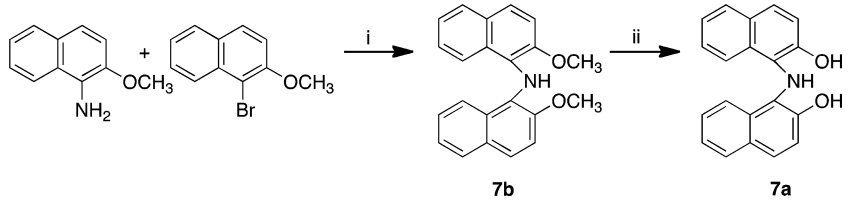
Scheme 3[a]



[a]Reagents and conditions: (i) BBr_3, CH_2Cl_2, −45 °C, 1 h; (ii) BBr_3, CH_2Cl_2, rt, 15 h; (iii) NaOH, THF/MeOH 1:1, reflux; (iv) BBr_3, CH_2Cl_2, rt, 35 min.

Scheme 4[a]



[a]Reagents and conditions: (i) $Pd(OAc)_2$, (±)-BINAP, Cs_2CO_3, 80 °C; (ii) BBr_3, CH_2Cl_2, −45 °C.

out with a pump/autosampler Waters (Alliance model 2695), a UV photodiode array detector Waters (model 2996), and a system data management Waters (Empower 2). The column used was generally Suplex pkb-100 (250 mm × 4.6 mm, 5 μm). Infrared (IR) spectra (Nujol mulls) were recorded on a Perkin-Elmer Spectrum-One spectrophotometer. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AC 400 Ultrashield 10 spectrophotometer (400 MHz) and on Varian Gemini 200 and Varian XL 300 spectrometers (200 and 300 MHz, respectively). The LC−MS analyses have been performed with instrumentation equipped with HPLC−MS Thermo-Finnigan Surveyor LC pump, PDA detector, and single quadrupole MSQ detector. Dimethylsulfoxide-d_6, 99.9% (code 44,139-2), and deuterochloroform, 98.8% (code 41,675-4), of isotopic purity (Aldrich) were used as NMR solvents. Column chromatographies were performed on silica gel (Merck, 70−230 mesh). All compounds were routinely checked by TLC by using aluminum-baked silica gel plates (Fluka DC-Alufolien Kieselgel 60 F254). Plates were visualized by UV light. Solvents were reagent grade and, when necessary, purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of rotary evaporator (Büchi) operating at a reduced pressure (~20 Torr). Organic solutions were dried over anhydrous sodium sulfate (Merck).

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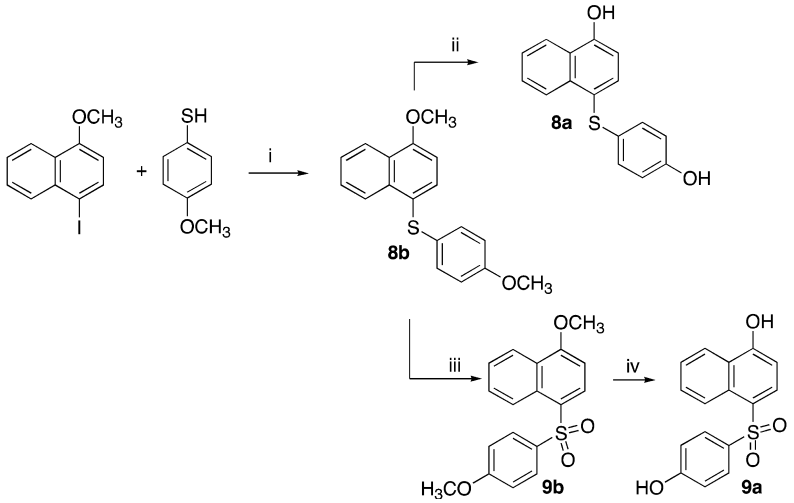
Scheme 5[a]



[a]Reagents and conditions: (i) Pd_2dba_3, DPEphos, *t*-BuOK, 100 °C; (ii) BBr_3, THF, −45 °C, 2 h; (iii) Oxone, water, rt, 16 h; (iv) BBr_3, THF, 20 h, rt.

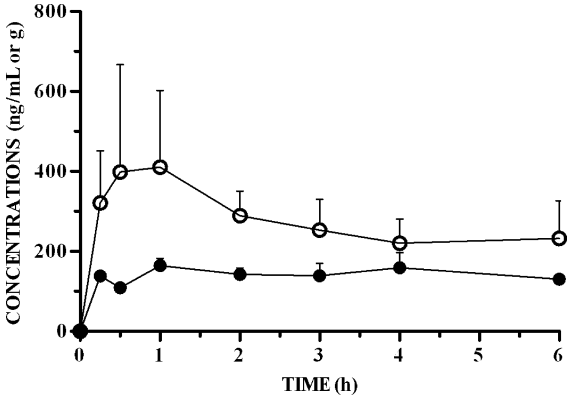


Figure 4. Mean plasma and brain concentration−time curves of compound **4q** after 25 mg/kg subcutaneous injection in mice. Each value is the mean ± SD of three determinations for plasma (plain circles) and brain (empty circles).

Analyses. 4a,9a-Dihydro-3,4-dihydroxy-1-[1-(4-sulfophenylamino)]-anthracene-9,10-dione (**2**) isolated by mixture **1** (Aldrich, mordant black 1324-21-6) was analyzed by LC−MS. The major component showed negative ion ESI-MS: calcd for $C_{20}H_{12}NO_7S$ [M − 1] 410.4, found 409.6.

A chromatographic separation was done on a semipreparative column Waters Xterra C18, 10 mm × 100 mm, 5 μm, under reversed phase conditions with a mobile phase consisting of A ($AcONH_4$ 10 mM/CH_3CN 80/20), B ($AcONH_4$ 10 mM/CH_3CN 60/40) under gradient conditions from 100% to 0% of A in 20 min, delivered at a flow rate of 1.00 mL/min, with ultraviolet detection at 280 nm. The column temperature was set at 25 °C, and the main component of the mixture ($C_{20}H_{12}NO_7S$ [M − 1] 410.4) was isolated, **2**. ^1H NMR (DMSO-d_6) δ 6.58 (s, 1H, anthracene C2−H), 7.31 (d, 2H, J_o = 10.8 Hz, benzene H), 7.63 (d, 2H, J_o = 10.8 Hz, benzene H), 7.89 (t, 2H, J_o = 9.6 Hz, anthracene C6−H and anthracene C7−H), 8.25 (dd, 2H, J_o = 9.6 Hz, anthracene C5−H and anthracene C8−H), 9.19 (s, 1H, NH). Anal. ($C_{20}H_{13}NO_7S$) C, H, N, S.

Syntheses. General Procedure for the Synthesis of *N*-Arylnaphthylamines 4b,m,o−r,t, 5c, 6d,f,g, and 7b. A dried flask was purged with argon, charged with (±)-BINAP (0.11 mmol), and capped with rubber septum. The flask was purged with argon again, and toluene (9.7 mL) was added. The mixture was heated to 80 °C under stirring until BINAP dissolved (1 min). The solution was cooled to room temperature, and $Pd(OAc)_2$ (0.07 mmol) was added. The flask was then purged with argon for 30 s. After the mixture was stirred at room temperature for 1 min, a solution of substituted naphthylamine (3.5 mmol) and appropriate aryl halide in toluene (1.5 mL) was added. Then Cs_2CO_3 (4.0 mmol) was introduced within the mixture which was then diluted with toluene (7 mL) and then purged with argon again. The mixture was heated to 80 °C under stirring and was cooled to room temperature before being diluted with ether, filtered, and concentrated. The crude product obtained was purified by column chromatography. Reagents, reaction time, eluent of chromatography, yield (%), melting point (°C), recrystallization solvent, IR, ^1H NMR, and analytical data are reported for each of the following compounds.

*Methyl 4-(1-Naphthylamino)benzoate (**4b**).* 1-Naphthalenamine and methyl 4-bromobenzoate; 16 h; chloroform/petroleum ether 3:1; 96%; 130−132 °C; toluene. IR ν 3340 (NH), 1694 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.79 (s, 3H, CH_3), 6.95 (m, 2H, benzene C3−H and C5−H), 7.45−7.60 (m, 4H, naphthalene H), 7.73 (m, 1H, naphthalene H), 7.79 (m, 2H, benzene C2−H and C6−H), 7.98 (m, 1H, naphthalene H), 8.06 (m, 1H, naphthalene H), 8.88 (s broad, 1H, NH). Anal. ($C_{18}H_{15}NO_2$) C, H, N, O.

*4-Methoxy-N-phenyl-1-naphthalenamine (**4m**).* 4-Methoxy-1-naphthalenamine[31] and bromobenzene; 21 h; ethyl acetate/*n*-hexane 1:1; 70%; 141−143 °C; cyclohexane/*n*-hexane. IR ν 3400 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.07 (s, 3H, CH_3), 5.75 (s broad, 1H, NH), 6.75−6.90 (m, 4H, naphthalene H and benzene C2−H and C6−H), 7.15−7.25 (m, 3H, benzene C3−H, C4−H, and C5−H), 7.50−7.60 (m, 2H, naphthalene H), 8.04 (m, 1H, naphthalene H), 8.33 (m, 1H, naphthalene H). Anal. ($C_{17}H_{15}NO$) C, H, N.

*Methyl 4-[(4-Methoxynaphthalen-1-yl)amino]benzoate (**4o**).* 4-Methoxy-1-naphthalenamine[31] and methyl 4-bromobenzoate; 24 h; ethyl acetate/*n*-hexane 1:1; 77%; 168−170 °C; benzene. IR ν 3300 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.89 (s, 3H, $COOCH_3$), 4.08 (s, 3H, OCH_3), 6.03 (s broad, 1H, NH), 6.45−6.71 (m, 2H, benzene C3−H and C5−H), 6.85 (d, J_o = 8.1 Hz, naphthalene H), 7.39 (d, J_o = 8.1 Hz, naphthalene H), 7.48−7.61 (m, 2H, naphthalene H), 7.85−7.96 (m, 3H, naphthalene H and benzene C2−H and C6−H), 8.35−8.41 (m, 1H, naphthalene H). Anal. ($C_{19}H_{17}NO_3$) C, H, N, O.

*N-(4-Methoxy-1-naphthyl)-N-(2-methoxyphenyl)amine (**4p**).* 4-Methoxy-1-naphthalenamine[31] and 2-methoxy-1-bromobenzene; 39 h; ethyl acetate/*n*-hexane 1:2; 70%; 108−110 °C; cyclohexane. IR ν 3395 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.98 and 4.02 (2s, 6H, CH_3), 6.60 and 6.91 (2 m, 2H, benzene C3−H and C6−H), 6.73 (m, 2H, benzene C4−H and C5−H), 6.80 (d, 1H, J_o = 8.1 Hz, naphthalene C2−H), 7.35 (d, 1H, J_o = 8.1 Hz, naphthalene C3−H), 7.48 (m, 2H, naphthalene C6−H and C7−H), 7.98 and 8.03 (2 m, 2H, naphthalene C5−H and C8−H). Anal. ($C_{18}H_{17}NO_2$) C, H, N.

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*4-Methoxy-N-(4-methoxyphenyl)-1-naphthalenamine (**4q**).* 4-Methoxy-1-naphthalenamine[31] and 4-methoxy-1-bromobenzene; 21 h; ethyl acetate/*n*-hexane 1:1; 96%; oil. IR ν 3380 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.81 and 4.04 (2s, 6H, CH_3), 5.90 (s broad, 1H, NH), 6.74−6.85 (m, 6H, naphthalene C2−H and C3−H and benzene H), 7.51−7.58 (m, 2H, naphthalene H), 8.04 (m, 1H, naphthalene H), 8.35 (m, 1H, naphthalene H). Anal. ($C_{18}H_{17}NO_2$) C, H, N.

*N-(5-Iodo-2-methoxyphenyl)-N-(4-methoxy)-1-naphthylamine (**4r**).* 4-Methoxy-1-naphthalenamine[31] and 2,4-diiodoanisole; 48 h; flash chromatography ethyl acetate/*n*-hexane 1:20; 18%; oil. IR ν 3390 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.95 and 4.03 (2s, 6H, CH_3), 6.61 (d, 1H, J_o = 8.1 Hz, benzene C3−H), 6.78 (d, 1H, J_m = 2.0 Hz, benzene C6−H), 6.82 (d, 1H, J_o = 8.1 Hz, naphthalene C3−H), 7.01 (dd, 1H, J_o = 8.3 Hz, J_m = 2.0 Hz benzene C4−H), 7.34 (d, 1H, J_o = 8.1 Hz, naphthalene C2−H), 7.50 (m, 2H, naphthalene C6−H and C7−H), 7.92 and 8.31 (2 m, 2H, naphthalene C5−H and C8−H). Anal. ($C_{18}H_{16}NO_2I$) C, H, N, I.

*Methyl 2-(Methoxymethyl)-5-[(4-methoxynaphthalen-1-yl)-amino]benzoate (**4t**).* 4-Methoxy-1-naphthalenamine[31] (9.1 mmol) and methyl 2-methoxy-5-bromobenzoate using (±) BINAP (0.76 mmol), $Pd(OAc)_2$ (0.51 mmol); 120 °C; 24 h; ethyl acetate/*n*-hexane 1:1; 97%; oil. IR ν 3320 (NH), 1695 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.88 (2s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 5.60 (s broad, 1H, NH), 6.79 (d, 1H, J = 8.2 Hz, naphthalene H), 6.88 (m, 2H, benzene C5−H and C6−H), 7.22 (d, 1H, J = 8.2 Hz, naphthalene H), 7.34 (m, 1H, benzene C2−H), 7.50−7.58 (m, 2H, naphthalene H), 7.99 and 8.34 (2 m, 2H, naphthalene H).

*Methyl 2-[(2-Methoxy-1-naphthyl)amino]benzoate (**5c**).* 2-Methoxy-1-naphthalenamine[32] and methyl-2-bromobenzoate; 15.5 h; ethyl acetate/*n*-hexane 1:5; 100%; 144−146 °C. IR ν 3321 (NH), 1681 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.86 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 6.09 (m, 1H, benzene H), 6.66 (m, 1H, benzene H), 7.18 (m, 1H, naphthalene H), 7.35−7.45 (m, 2H, benzene H and naphthalene H), 7.57 (m, 1H, naphthalene H), 7.68 (m, 1H, benzene H), 7.88−7.95 (m, 3H, naphthalene H), 9.17 (s broad, 1H, NH). Anal. ($C_{19}H_{17}NO_3$) C, H, N.

*(1-Methoxy-2-naphthyl)phenylamine (**6d**).* 1-Methoxy-2-naphthalenamine[33] and bromobenzene; 16 h; ethyl acetate/*n*-hexane 1:1; 83%; 43−45 °C; *n*-hexane. IR ν 3395 (NH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.80 (s, 3H, CH_3), 6.85 (m, 1H, benzene H), 7.07−7.65 (m, 8H, naphthalene H and benzene H), 7.84 (m, 1H, naphthalene H), 7.93 (s broad, 1H, NH), 7.99 (m, 1H, naphthalene H). Anal. ($C_{17}H_{15}NO$) C, H, N.

*Methyl 4-(1-Methoxy-2-naphthylamino)benzoate (**6f**).* 1-Methoxy-2-naphthalenamine[33] and methyl 4-bromobenzoate; 4 h and 10 min; chloroform/ethyl acetate 9:1; 97%; 153−154 °C; ligroin. IR ν 3327 (NH), 1691 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.95 (s, 3H, CH_3), 7.14 (d, 2H, J_o = 8.8 Hz, benzene C2−H and C6−H), 7.44−7.48 (m, 2H, naphthalene H), 7.55−7.59 (m, 1H, naphthalene H), 7.64−7.69 (m, 2H, naphthalene H), 8.03 (d, 2H, J_o = 8.8 Hz, benzene C3−H and C5−H), 8.10 (m, 1H, naphthalene H). Anal. ($C_{19}H_{17}NO_3$) C, H, N.

*N-(4-Iodophenyl)-1-methoxynaphthalen-2-amine (**6g**).* 1-Methoxy-2-naphthalenamine[33] and 1,4-diiodobenzene; 19 h; 37%; 83−84 °C; *n*-hexane. IR ν 3327 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.96 (s, 3H, CH_3), 6.97 (d, 2H, J_o = 8.8 Hz, benzene C2−H and C6−H), 7.40−7.43 (m, 1H, naphthalene H), 7.53−7.57 (d, 2H, J_o = 8.8 Hz, benzene C3−H and C5−H), 7.61−7.74 (m, 3H, naphthalene H), 7.83−7.85 (m, 1H, naphthalene H), 8.06−8.08 (m, 1H, naphthalene H). Anal. ($C_{19}H_{14}NIO$) C, H, N, I.

*2-Methoxy-N-(2-methoxy-1-naphthyl)naphthalen-1-amine (**7b**).* 2-Methoxy-1-naphthalenamine[32] and 2-methoxy-1-bromonaphthalene; ethyl acetate/*n*-hexane 1:5; 59%; oil. IR ν 3380 (NH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.55 (s, 6H, CH_3), 7.05 (s broad, 1H, NH), 7.20−7.38 (m, 6H, naphthalene H), 7.58 (m, 2H, naphthalene H), 7.81−7.93 (m, 4H, naphthalene H). Anal. ($C_{22}H_{20}NO_2$) C, H, N.

General Procedure for the Synthesis of *N*-Arylnaphthylamines 4c−f. A dried flask was purged with argon, charged with (±)-BINAP (0.11 mmol), and capped with rubber septum. The flask was purged with argon again, and toluene (9.7 mL) was added. The mixture was heated to 80 °C under stirring until the BINAP was dissolved (1 min). The solution was cooled to room temperature, and $Pd(OAc)_2$ (0.07 mmol) was added. The flask was then purged with argon for 30 s. After stirring of the mixture at room temperature for 1 min, a solution of 4-halo-1-substituted naphthalene (3.5 mmol) with appropriate 4-substituted aniline (2.9 mmol) in toluene (1.5 mL) was added. Cs_2CO_3 (4.0 mmol) was introduced within the mixture. Toluene (7 mL) was added and then the flask purged with argon again. The mixture was heated to 80 °C under stirring, then was cooled to room temperature, diluted with ether, filtered, and concentrated. The crude product that was obtained was purified by column chromatography to obtain the desired adduct. Reagents, reaction time, eluent of chromatography, yield (%), melting point (°C), recrystallization solvent, IR, ^1H NMR, and analytical data are reported for each of the following compounds.

*4-Fluoro-N-(4-fluorophenyl)naphthalen-1-amine (**4c**).* 4-Fluoroaniline and 1-bromo-4-nitronaphthalene; 5 h and 45 min; chloroform; 91%; 62−64 °C; *n*-hexane. IR ν 3395 (NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.55 (s broad, 1H, NH), 6.89−6.90 (m, 2H, benzene H), 6.98−7.03 (m, 2H, benzene H), 7.11−7.17 (m, 1H, naphthalene C2−H), 7.24 (m, 1H, naphthalene C3−H), 7.57−7.66 (m, 2H, naphthalene C6−H and C7−H), 8.05 and 8.20 (2 m, 2H, naphthalene C5−H and C8−H). Anal. ($C_{16}H_{11}NF_2$) C, H, N, F.

*4-Fluoro-N-[4-(methylthio)phenyl]naphthalen-1-amine (**4d**).* 1-(Methylthio)aniline and 1-bromo-4-fluoronaphthalene; 17 h; chloroform/petroleum ether 1:1; 94%; 71−72 °C; *n*-hexane. IR ν 3337 (NH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, SCH_3), 6.93 (d, 2H, J_o = 8.7 Hz, benzene C3−H and C5−H), 7.20−7.33 (d, 2H, J_o = 8.7 Hz, benzene C2−H and C3−H), 7.63−7.71 (m, 2H, naphthalene C6−H and C7−H), 8.07 and 8.18 (2 m, 2H, naphthalene C5−H and C8−H), 8.21 (s broad, 1H, NH). Anal. ($C_{17}H_{14}NFS$) C, H, N, F, S.

*N-(4-Methoxyphenyl)-4-nitronaphthalen-1-amine (**4e**).* 4-Iodo-1-nitronaphthalene[34] and 4-methoxyaniline; chloroform; 15 h; 38%; oil. IR ν 3365 (NH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.93 (s, 3H, CH_3), 6.85 (s broad, 1H, NH), 6.80 (m, 1H, naphthalene H), 7.05 (d, 2H, J_o = 8.8 Hz, benzene C3−H and C5−H), 7.30 (d, 2H, J_o = 8.8 Hz, benzene C2−H and C6−H), 7.67−7.81 (2 m, 2H, naphthalene C6−H and C7−H), 8.08, 8.39, and 9.07 (3 m, 3H, naphthalene C2−H, C5−H, and C8−H). Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

*N,N-Dimethyl-N′-[4-(methylthio)phenyl]naphthalene-1,4-diamine (**4f**).* 4-(Methylthio)aniline and 1-bromo-4-(dimethylamino)-naphthalene; 16 h; chloroform; 81%; oil. IR ν 3381 (NH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.42 (s, 3H, SCH_3), 2.84 (s, 6H, NCH_3), 6.86 (d, 2H, J_o = 8.8 Hz, benzene C2−H and C6−H), 7.14−7.20 (m, 3H, J_o = 8.8 Hz, naphthalene H and benzene C3−H and C5−H), 7.56 (m, 2H, naphthalene C2−H and C3−H), 8.07−8.09 (m, 2H, NH and naphthalene H), 8.23 (m, 1H, naphthalene H). Anal. ($C_{19}H_{20}NS$) C, H, N, S.

*1-Methoxy-4-[(4-methoxyphenyl)thio]naphthalene (**8b**).* A dried flask was charged with Pd_2dba_3 (130 mg, 0.141 mmol), dissolved in degassed toluene (115 mL), treated with DPEphos (150 mg, 0.28 mmol), and purged with argon. The mixture was stirred at room temperature for 3 min. Then 1-methoxy-4-iodonaphthalene (4.0 g, 14.1 mmol) and 4-methoxythiophenol (2.0 g, 14.1 mmol) were added under argon atmosphere. *t*-BuOK (1.7 g, 15.5 mmol) was added and the flask purged with argon. The mixture was stirred for 2 h at 100 °C, cooled to room temperature, and filtered on a Celite cake, and the filtrate was concentrated in vacuo. The crude product (6.2 g) was purified by column chromatography (*n*-hexane/acetone 10:1) to obtain the desired adduct as a slightly colored (3.6 g) solid, which was purified by recrystallization (*n*-hexane) to obtain 2.7 g of pure **8b** (65%): mp 83−85 °C. IR ν 2937 (CH) cm^{-1}. ^1H NMR (acetone-d_6) δ 3.78 (s, 3H, CH_3), 4.10 (s, 3H, CH_3), 6.88 (d, 2H, J_o = 8.86 Hz, benzene H), 7.03 (d, 1H, J_o = 8.01 Hz, naphthalene C2−H), 7.20 (d, 2H, J_o = 8.86 Hz, benzene H), 7.57−7.64 (m, 2H, naphthalene C6−H and C7−H), 7.75 (d, 1H, J_o = 8.01 Hz, naphthalene C3−H), 8.34−8.40 (m, 2H, naphthalene C5−H and C8−H). Anal. ($C_{18}H_{16}O_2S$) C, H, N, S.

General Procedure for the Synthesis of Acids 4a,n,s and 6e. A solution of the appropriate methyl ester (1.5 mmol) and 1 N NaOH

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(3.7 mL) in THF/ethanol 1:1 (20 mL) was refluxed for 3 h for **4a**, 3 h and 30 min for **4n,t**, and 1 h and 40 min for **6e**, while stirring. Then the mixture was poured into crushed ice and extracted with ethyl acetate (30 mL). The aqueous layer was treated with 1 N HCl until pH 3 and then extracted with ethyl acetate (3 × 50 mL). The organic phases were collected, washed with brine (3 × 100 mL), dried, and the solvent was removed under reduced pressure to yield the final product. Reagents, reaction time, eluent of chromatography, yield (%), melting point (°C), recrystallization solvent, IR, ^{1}H NMR, and analytical data are reported for each of the following compounds

*4-(1-Naphthylamino)benzoic Acid (**4a**).* **4b**; 3 h; 59%; 227−229 °C; toluene. IR ν 3390 (NH), 2900 (OH), 1670 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 6.95 (m, 2H, benzene C3−H and C5−H), 7.46−7.60 (m, 4H, naphthalene H), 7.72 (m, 1H, naphthalene H), 7.78 (m, 2H, benzene C2−H and C6−H), 7.96 (m, 1H, naphthalene H), 8.07 (m, 1H, naphthalene H), 8.81 (s broad, 1H, NH), 12.29 (s broad, 1H, OH). Anal. ($C_{17}H_{13}NO_2$) C, H, N.

*4-(4-Methoxy-1-naphthylamino)benzoic Acid (**4n**).* **4o**; 3.5 h; 50%; 153−154 °C; isopropanol. IR ν 3400 (NH), 3000 (OH), 1650 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 4.01 (s, 3H, CH_3), 6.70 (d, 2H, J_o = 8.5 Hz, benzene C3−H and C5−H), 7.01 (d, 1H, J = 8.0 Hz, naphthalene C3−H), 7.39 (d, 1H, J = 8.0 Hz, naphthalene C2−H), 7.55 (m, 2H, naphthalene C6−H and C7−H), 7.71 (d, 2H, J_o = 8.5 Hz, benzene C2−H and C6−H), 7.90 (m, 1H, naphthalene H), 8.20 (m, 1H, naphthalene H), 8.54 (s broad, 1H, NH). Anal. ($C_{18}H_{15}NO_3$) C, H, N.

*2-Methoxy-5-(4-methoxy-1-naphthylamino)benzoic Acid (**4s**).* **4t**; 3.5 h; 50%; 153−154 °C; isopropanol. IR ν 3300 (NH), 3160 (OH), 1690 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 3.76 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 6.91−6.98 (m, 3H, naphthalene H and benzene C3−H and C4−H), 7.16 (m, 1H, benzene C6−H), 7.21 (d, 1H, naphthalene H), 7.53 (m, 2H, naphthalene H), 7.76 (s broad, 1H, NH), 8.05 and 8.20 (2 m, 2H, naphthalene H), 12.50 (s broad, 1H, OH). Anal. ($C_{19}H_{17}NO_4$) C, H, N.

*4-(1-Methoxy-2-naphthylamino)benzoic Acid (**6e**).* **6f**; 1 h and 40 min; 100%; 233−235 °C; ethanol. IR ν 3403 (OH and NH), 1691 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H, CH_3), 7.01 (d, 2H, J_o = 8.6 Hz, benzene C2−H and C6−H), 7.46−7.59 (m, 3H, naphthalene H), 7.72 (m, 1H, naphthalene H), 7.82 (d, 2H, J_o = 8.6 Hz, benzene C3−H and C5−H), 7.93 (m, 1H, naphthalene H), 8.08 (m, 1H, naphthalene H), 8.59 (s broad, 1H, NH), 12.32 (s broad, 1H, COOH). Anal. ($C_{18}H_{15}NO_3$) C, H, N.

General Procedure for the Synthesis of Compounds 4g,i−l, 5a,b, 6a−c, 7a, 8a, and 9a. A solution of the proper *N*-phenyl-1-naphthalenamine derivative **4m,o,q,t**, **5c**, **6d,f**, **7b**, **8b**, or **9b** (2.4 mmol) in dichloromethane (27 mL) was added dropwise to 1 M BBr_3 (12.6 mL, 12.6 mmol) in the same solvent at −45 °C under argon atmosphere while stirring. After being diluted with water (50 mL), the mixture was extracted with ethyl acetate (3 × 50 mL) and the organic phases were collected, washed with brine (3 × 100 mL), and dried. Evaporation of the solvent gave a crude product, which was chromatographed to afford pure product(s). Substrate, reaction temperature, reaction time, eluent of chromatography, yield (%), melting point (°C), recrystallization solvent, IR, ^{1}H NMR, and analytical data are reported for each of the following compounds.

*4-Phenylamino-naphthalen-1-ol (**4g**).* **4m**; −45 °C under argon atmosphere; 15 h; ethyl acetate/*n*-hexane 1:3; 88%; oil. IR ν 3350 (OH, NH) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 5.30 and 5.65 (2s,broad, 2H, OH and NH), 6.75−6.87 (m, 4H, naphthalene H and benzene C2−H and C6−H), 7.15−7.30 (m, 3H, benzene C3−H, C4−H, and C5−H), 7.50−7.57 (m, 2H, naphthalene H), 8.50 (m, 1H, naphthalene H), 8.25 (m, 1H, naphthalene H). Anal. ($C_{16}H_{13}NO$) C, H, N.

*4-(4-Hydroxy-1-naphthylamino)benzoic Acid (**4i**).* **4o**; rt; 15 h; ethyl acetate/*n*-hexane 9:2; 45%; 214−217 °C; toluene. IR ν 3360 (OH, NH), 2800 (COOH) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 6.63 (d, 2H, J_o = 8.7 Hz, benzene C3−H and C5−H), 6.92 (d, 1H, J = 8.0 Hz, naphthalene H), 7.27 (d, 1H, J = 8.0 Hz, naphthalene H), 7.50 (m, 2H, naphthalene H), 7.69 (d, 2H, J_o = 8.7 Hz, benzene C2−H and C6−H), 7.85 (m, 1H, naphthalene H), 8.20 (m, 1H, naphthalene H), 8.45 (s broad, 1H, NH), 10.20 (s broad, 1H, OH), 12.15 (s broad, 1H, COOH). Anal. ($C_{17}H_{13}NO_3$) C, H, N.

*Methyl 4-(4-Hydroxy-1-naphthylamino)benzoate (**4j**).* **4o**; −45 °C; 1 h; ethyl acetate/*n*-hexane 1:2; 58%; 188−190 °C; benzene/*n*-hexane. IR ν 3377 (OH, NH), 1680 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 3H, CH_3), 6.60−6.67 (m, 2H, benzene C3−H and C5−H), 6.86−6.88 (d, 1H, J = 8.0 Hz, naphthalene H), 7.21−7.23 (d, 1H, J = 8.0 Hz, naphthalene H), 7.44−7.48 (m, 2H, naphthalene H), 7.65−7.67 (m, 2H, benzene C2−H and C6−H), 7.82 (m, 1H, naphthalene H), 8.17 (m, 1H, naphthalene H), 8.50 (s broad, 1H, NH), 10.18 (s broad, 1H, OH). Anal. ($C_{18}H_{15}NO_3$) C, H, N.

*4-(4-Hydroxy-phenylamine)naphthalen-1-ol (**4k**).* The solvent was purged with argon. **4q**; −45 °C; 15 h; ethyl acetate/*n*-hexane 1:1; 80%; 74 °C (dec). IR ν 3375 (OH, NH) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 6.55−7.00 (m, 7H, naphthalene H, benzene H and NH), 7.38−7.47 (m, 2H, naphthalene H), 7.95−8.13 (m, 2H, naphthalene H), 8.67 and 9.67 (2s broad, 2H, OH). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

*2-Hydroxy-5-(4-hydroxy-1-naphthylamino)benzoic Acid (**4l**).* **4t**; rt; 15 h; ethyl acetate; 17%; 175 °C (dec). IR ν 3350 (OH, NH), 3000 (COOH), 1635 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 6.73 (d, 1H, J_o = 8.7 Hz, benzene C3−H), 6.82 (d, 1H, J = 8.0 Hz, naphthalene H), 6.97 (dd, 1H, J_o = 8.7 Hz, J_m = 2.7 Hz, benzene C4−H), 7.05 (d, 1H, J = 8.0 Hz, naphthalene H), 7.20 (d, 1H, J_m = 2.7 Hz, benzene C6−H), 7.44−7.49 (m, 2H, naphthalene C6−H and C7−H), 7.99 (m, 1H, naphthalene H), 8.15 (m, 1H, naphthalene H), 9.85 (3s broad, 3H, OH, COOH and NH). Anal. ($C_{17}H_{13}NO_4$) C, H, N.

*2-(2-Hydroxy-1-naphthylamino)benzoic Acid (**5a**) and Methyl 2-(2-Hydroxy-1-naphthylamino)benzoate (**5b**).* **5c**; rt; 23 min; ethyl acetate/*n*-hexane 1:2. **5a**: 55%; 215−216 °C; toluene. IR ν 3361 (OH and COOH), 3325 (NH), 1659 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 6.09 (m, 1H, benzene H), 6.62 (m, 1H, benzene H), 7.14 (m, 1H, naphthalene H), 7.28−7.31 (m, 2H, naphthalene H), 7.39 (m, 1H benzene H), 7.62 (m, 1H, benzene H), 7.75 (m, 1H, naphthalene H), 7.83−7.89 (m, 2H, naphthalene H), 9.28 (s broad, 1H, NH), 12.86 (s broad, 1H, COOH). Anal. ($C_{17}H_{13}NO_3$) C, H, N. **5b**: 45%; 157−158 °C; cyclohexane. IR ν 3407 (OH and COOH), 3319 (NH), 1681 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 3.88 (s, 3H, CH_3), 6.10 (m, 1H, benzene H), 6.64 (m, 1H, benzene H), 7.17 (m, 1H, naphthalene H), 7.28−7.31 (m, 2H, naphthalene H), 7.39 (m, 1H benzene H), 7.62 (m, 1H, benzene H), 7.77 (m, 1H, naphthalene H), 7.84−7.95 (m, 2H, naphthalene H), 9.05 (s broad, 1H, NH), 9.78 (s broad, 1H, OH). Anal. ($C_{18}H_{15}NO_3$) C, H, N.

*1-Hydroxy-N-phenylnaphthalen-2-amine (**6a**).* **6d**; −45 °C; 30 min; ethyl acetate/*n*-hexane 1:3; 88%; >300 °C. IR ν 3150 (NH and OH) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 6.70 (m, 1H, benzene H), 6.81−6.88 (m, 2H, benzene H), 7.07−7.16 (m, 2H, naphthalene H), 7.31−7.42 (m, 4H, benzene H and naphthalene H), 7.77 (m, 1H, naphthalene H), 8.11 (m, 1H, naphthalene H). Anal. ($C_{16}H_{13}NO$) C, H, N.

*4-(1-Hydroxy-2-naphthylamino)benzoic Acid (**6b**) and Methyl 4-(1-Hydroxy-2-naphthylamino)benzoate (**6c**).* **6f**; rt; 35 min; ethyl acetate/*n*-hexane 1:1. **6b**: 45%; 210 °C (dec); methanol. IR ν 3426 (OH and COOH), 3353 (NH), 1654 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 6.78−6.80 (m, 2H, benzene C2−H and C6−H), 7.33−7.36 (m, 1H, naphthalene H), 7.42−7.50 (m, 3H, naphthalene H), 7.74−7.77 (m, 2H, benzene C3−H and C5−H), 7.84−7.86 (m, 1H, naphthalene H), 8.18 (s broad, 1H, NH), 8.19−8.21 (m, 1H, naphthalene H), 9.40 (s broad, 1H, OH), 12.20 (s broad, 1H, COOH). Anal. ($C_{17}H_{13}NO_3$) C, H, N. **6c**: acetone/*n*-hexane 1:4; 55%;175−176 °C; toluene. IR: ν 3334 (OH and NH) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3H, CH_3), 7.28−7.31 (m, 2H, naphthalene H), 7.35 (m, 1H, naphthalene H), 7.39 (m, 2H, J_o = 8.8 Hz, benzene C2−H and C6−H), 7.45−7.51 (m, 3H, naphthalene H), 7.77 (m, 2H, J_o = 8.8 Hz, benzene C3−H and C5−H), 8.25 (s broad, 1H, NH), 9.40 (s broad, 1H OH). Anal. ($C_{18}H_{15}NO_3$) C, H, N.

*2-Hydroxy-N-(2-hydroxy-1-naphthyl)naphthalen-1-amine (**7b**).* **7a**; −45 °C, rt; 15 h, ethyl acetate/*n*-hexane 1:2. After isolation the compound **7a** rapidly degraded so that no analytical and structural data are available.

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*4-[(4-Hydroxyphenyl)thio]-1-naphthol (**8a**).* **8b**; −45 °C, 15.5 h, then rt; 7 h; ethyl acetate/*n*-hexane 2:5; 61%; 161−163 °C; toluene. IR ν 3255 (OH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 6.70 (d, 2H, J_o = 8.69 Hz, benzene H), 6.93 (d, 1H, naphthalene C2−H), 7.06 (d, 2H, J_o = 8.69 Hz, benzene H), 7.51−7.62 (m, 3H, naphthalene C3−H, C6−H, and C7−H), 8.23 and 8.27 (2 m, 2H, naphthalene C5−H and C8−H), 9.52 and 10.61 (2s broad, 2H, OH). Anal. ($C_{16}H_{12}O_2S$) C, H, S.

*4-[(4-Hydroxyphenyl)sulfonyl]-1-naphthol (**9a**).* **9b**; −45 °C, 20 min, then rt; 20 h; ethyl acetate/chloroform 1:2; 58%; 203−205 °C; toluene. IR ν 3300 (OH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.90 (d, 2H, J_o = 8.77 Hz, benzene H), 7.07 (d, 1H, J_o = 8.29 Hz, naphthalene C2−H), 7.58 and 7.67 (2 m, 2H, naphthalene C6−H and C7−H), 7.77 (d, 2H, J_o = 8.77 Hz, benzene H), 8.28−8.31 (m, 2H, naphthalene C5−H and C8−H), 8.48 (d, 1H, J_o = 8.29 Hz, naphthalene C3−H), 10.54 and 11.51 (2s broad, 2H, OH). Anal. ($C_{16}H_{12}O_4S$) C, H, S.

*1-Methoxy-4-[(4-methoxyphenyl)sulfonyl]naphthalene (**9b**).* **8b** (1 g, 3.4 mmol) was dissolved in methanol (84 mL). At 0 °C a solution of Oxone (6.3 g, 10.2 mmol) in water (20 mL) was added. The mixture was stirred for 16 h at room temperature. The mixture was poured into water, extracted with ethyl acetate (3 × 100 mL), and the collected organic layers were washed with brine (3 × 100 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuum, obtaining a crude product which was purified by column chromatography (chloroform as eluent) affording the pure product **9b** (82%): mp 165−167 °C; toluene. IR ν 2900 (CH) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.82 (s, 3H, CH_3), 4.12 (s, 3H, CH_3), 7.11 (d, 2H, J_o = 8.69 Hz, benzene H), 7.25 (d, 1H, J_o = 8.47 Hz, naphthalene C2−H), 7.64 and 7.72 (2 m, 2H, naphthalene C6−H and C7−H), 7.90 (d, 2H, J_o = 8.69 Hz, benzene H), 8.30 (d, 1H, J_o = 8.47 Hz, naphthalene C3−H), 8.46−8.52 (m, 2H, naphthalene C5−H and C8−H). Anal. ($C_{18}H_{16}O_4S$) C, H, S.

*4-(4-Hydroxymethyl)phenylamino)naphthalen-1-ol (**4h**).* A solution of **4j** (350 mg, 1.2 mmol) in anhydrous THF (40 mL) was added dropwise into a 0 °C suspension of $LiAlH_4$ (230 mg, 6.0 mmol) in the same solvent (40 mL). After the addition, the mixture was stirred at room temperature for 15 h. The excess of $LiAlH_4$ was carefully destroyed with crushed ice, and the precipitate that formed was filtered on a Buchner apparatus. The solution was concentrated, extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (3 × 100 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum, obtaining 320 mg of crude product. Because it was unstable, it was immediately solubilized in 10 mL of methanol and added dropwise to a solution of HCl in methanol cooled at 0 °C, prepared by adding dropwise acetyl chloride (2.2 mmol) in methanol (10 mL). The mixture was stirred at 0 °C for 5 min after which anhydrous diethyl ether (100 mL) was added and the resulting precipitate was filtered to obtain 220 mg (33%) of hydrochloride **4h**, with mp > 300 °C.

Biological Assays. In Vitro Assays. Compounds **4a−t**, **5a−c**, **6a−g**, **7a,b**, **8a,b**, and **9a,b** (Table 1) were tested for their ability to inhibit aggregation of A$\beta_{(1-42)}$, via the binding of the ThT.

Preparation of the Nonaggregate A$\beta_{(1-42)}$. The A$\beta_{(1-42)}$ was dissolved in a mixture of acetonitrile and distilled water (CH_3CN/H_2O 1:1) to a final concentration of 1 mg/mL. The solution was divided in aliquots of 2 mL and stored at −80 °C until use. The working solution was prepared, diluting the stock solution with H_2O to get a final concentration of 44 μM.

Preparation of the Aggregate A$\beta_{(1-42)}$. The A$\beta_{(1-42)}$ was dissolved in a mixture of acetonitrile and distilled water (CH_3CN/H_2O 1:1) to the final concentration of 1 mg/mL. An aliquot of 2 mL was freeze-dried to eliminate the trifluoroacetic acid resulting from the peptide synthesis. The A$\beta_{(1-42)}$ peptide was subsequently dissolved in 0.1 mL of DMSO and 5.0 mL of 2× PBS, pH 7.4. Once dissolved, the A$\beta_{(1-42)}$ was incubated at 37 °C for 8 days. After sonication, it was diluted with 2× PBS to get a final concentration 17.4 μM. The aggregate A$\beta_{(1-42)}$ was divided in 20 aliquots and stored at −80 °C.

Screening of Inhibitory Activity of Aβ Aggregation (ThT). The drugs to be tested as fibrillogenesis inhibitor were dissolved in a minimum amount of DMSO and then diluted with water to the final concentrations of 10 or 100 μM, with a total percentage of DMSO inferior to 10%. The assays were performed in triplicate in 96-well plates. The compounds to be tested were added in wells containing the aggregate A$\beta_{(1-42)}$. Then after 15 min, the nonaggregate A$\beta_{(1-42)}$ was added. The 96-well plates were incubated at 37 °C under agitation for 24 h. The following day 200 μL of a solution containing 10 μM ThT and 50 μM Na_2HPO_4, pH 6.5, was added to each well. Fluorescence was measured in a VICTOR 2 (WALLAC) fluorescence spectrophotometer (λ_{ex} = 450 nm, λ_{em} = 486 nm).[33]

The data are expressed as percent of residual aggregated Aβ, and the dose reducing the aggregate formation of the 50% (IC_{50}) was estimated. The % of aggregation was determined by the following formula:

$$[(A\beta + \text{test compound}) - (\text{blank} + \text{test compound})] \times 100/[(\text{control} + A\beta) - \text{blank}]$$

Blood−Brain Barrier Crossing. Ten mice were given subcutaneously compound **4q** dissolved in 10% ethanol, 40% PEG-400, and 50% saline and were killed by decapitation under deep anesthesia in order to obtain information on the concentration achieved in brain and its relationship with plasma concentrations. Mixed arteriovenous trunk blood was collected at 0, 15, 30, 60, 120, 180, and 240 min after dosing and centrifuged at 3000*g* for 10 min, and the plasma was stored at −20 °C. Brain was removed immediately, blotted with paper to remove excess surface blood, and quickly frozen in dry ice.

Compound **4q** was extracted from plasma, and brain homogenate was obtained by a solid−liquid extraction procedure and quantified by reverse phase HPLC with UV detection (224 nm). To 0.3 mL of plasma were added 0.3 mL of CH_3CN/0.01 N phosphate buffer, pH 7.4 (70:30, v/v), and 0.1 mL of a solution of the internal standard. After centrifugation, the supernatants were added to Oasis HLB 1 cc cartridges which were prewetted with methanol and distilled water. Cartridges were washed with methanol−water (15:85 v/v) and then methanol only, interrupting the vacuum before completely drying the column. The compound was removed by eluting the cartridges with methanol (2.5 mL), and the collected fractions evaporated to dryness under nitrogen. The residue was dissolved in the mobile phase, centrifuged, and analyzed by HPLC. Brain tissue was homogenized (1 g/5 mL) in CH_3CN/0.001 M phosphate buffer, pH 7.4 (70:30, v/v), and a volume containing approximately 100 mg of tissue was centrifuged and processed as for plasma. Separation was done on a μBondapak C18 column protected by a LiChrosphere RP8 precolumn at room temperature. The mobile phase was $H_2O/CH_3CN/CH_3OH$/*n*-butanol (43:42:14:1, v/v), containing 65 mM ammonium acetate. The retention times were 10.5 min for **4q** and 20.5 for the internal standard.

Mean brain and plasma area under the concentration−time curve over the dosing interval (AUC_t) were determined using the linear trapezoidal rule. The maximum concentration (C_{max}) and the time (t_{max}) were read directly from the plasma and brain concentration time data.

■ ASSOCIATED CONTENT

Supporting Information

Results from elemental analyses of compounds **2**, **4a−g,i−s**, **5a−c**, **6a−g**, **7b**, **8a,b**, and **9a,b**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*For R.D.S.: phone, +39-06-49913150; fax, +39-06-49913133; e-mail, roberto.disanto@uniroma1.it. For P.M.: phone, +39-06-91393906; fax, +39-06-91393638; e-mail, Patrizia.Minetti@sigma-tau.it.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CR, Congo Red; IL, interleukin; ABBB, alizarin blue-black B; ThT, thioflavin T; BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl; Pd_2dba_3, tris(dibenzylideneacetone)dipalladium; DPEphos, bis(2-diphenylphosphinophenyl) ether

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