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Aβ Aggregation inhibitors. Part 1: Synthesis and biological activity of phenylazo benzenesulfonamides

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Abstract—Phenylazo benzenesulfonamides were designed and synthesized as β-amyloid ($A\beta_{40}$) fibril assembly inhibitors, and evaluated for inhibition of $A\beta_{40}$ aggregation and neurotoxicity using rat cortical neurons. Compound **2** (**LB-152**) was the most potent compound in this study, and the *para*-NMe_2 group on the end of the phenylazo moiety may play an important role in preventing $A\beta_{40}$ fibril formation. **LB-152** provides a new lead for further development of potential β-amyloid aggregation inhibitors to treat AD.

Alzheimer's disease (AD) neuropathology is characterized by extracellular amyloid deposition of senile plaques and neurofibrillary tangles in vulnerable AD brain regions.[1] Clinical symptoms of AD include cognitive decline, irreversible memory loss, disorientation, language impairment, etc. Because this devastating illness affects a large number of older patients and their families and no effective treatment or cure currently exists, better diagnosis and treatment of AD are urgently needed. Although the mechanism by which amyloid fibril formation is associated with AD dementia is not completely understood, recent research suggests that aggregation of the amyloid-β peptide (Aβ) into macromolecular β-sheet fibrils plays a causal role in the pathogenesis of AD.[2] Suppression of this transition from monomeric to polymeric Aβ aggregation is believed to be a singular therapy for AD.[3] Until now, much research has focused on the discovery and development of non-peptide small-molecule Aβ aggregation inhibitors for clinical evaluation in AD treatment.[4,5]

Small-molecule inhibitors from various chemical classes have recently been reported and reviewed.[6,7] These compounds inhibit or reduce in vitro aggregation of Aβ and are somewhat similar in structure to biphenyl naphthyl diazo dyes such as Congo red (CR) and related sulfonate anions, which reportedly disrupt Aβ aggregation and reduce Aβ toxicity.[8,9] Unfortunately, the therapeutic potential of CR is severely diminished by the fact that it does not cross the blood–brain barrier (BBB).[10] Also, a naphthyl monoazo derivative **1** (Fig. 1), a small-molecule probe for inhibition of $A\beta_{40}$ aggregation, has been extensively discussed.[11] These facts suggest that naphthylsulfonamide azo dyes can provide an appropriate starting point for future efforts to develop inhibitors of Aβ aggregation.[12]

In the process of seeking new leads for $A\beta_{40}$ aggregation inhibitors, we employed the concept of bioisosterism to design phenylazo benzenesulfonamide derivatives (Fig. 2). To our knowledge, no phenylazo benzenesulfonamide derivative has been reported to inhibit $A\beta_{40}$ aggregation; however, we previously reported the synthesis of a series of *trans*-stilbene benzenesulfonamide derivatives as potent antitumor agents.[13] Based on the principle of isosteric replacement,[14] the *C,C* double bond in the *trans*-stilbene benzenesulfonamides can be interchanged with the *N,N* double bond in phenylazo benzenesulfonamide derivatives. Consequently, the structure of the reference inhibitor **1** was also simplified by replacing the naphthylazo moiety with a phenylazo group. Thus,

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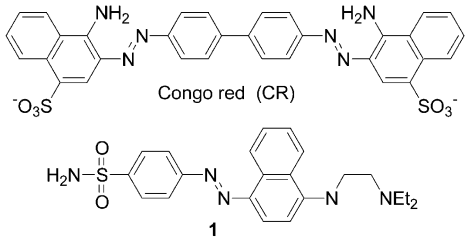


Figure 1.

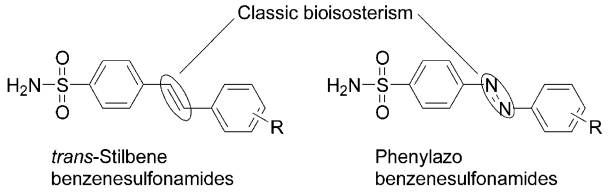
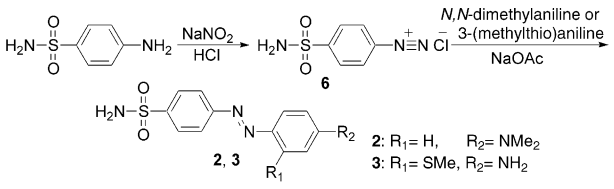


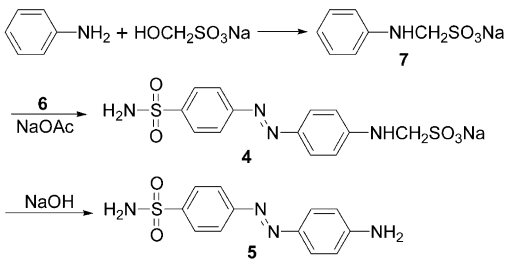
Figure 2.

new phenylazo benzenesulfonamides were synthesized and evaluated for $A\beta_{40}$ aggregation inhibitory activity and neurotoxicity.

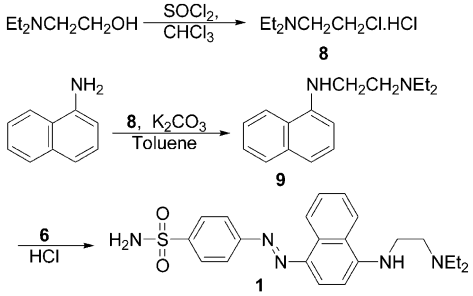
The synthesis of the target compounds (**1**–**5**) is outlined in Schemes 1–3. Although the synthesis of these derivatives has already been reported,[15] only a limited series of examples has been described, and neither the biological activity nor the possible chemical conversion was investigated. This process involved initial diazotization of sulfanilamide with sodium nitrite in hydrochloride solution to afford the diazonium salt **6**, followed by coupling with the molar equivalent of *N,N*-dimethylaniline or 3-(methylthio)aniline, in the presence of sodium acetate solution at 0 °C. The reactions were preferentially formed with a change in color from pale yellow to deep brown or reddish orange to afford the corresponding phenylazo benzenesulfonamide derivatives **2** and **3** (Scheme 1).



Scheme 1. Synthesis of phenylazo benzenesulfonamide derivatives **2** and **3**.



Scheme 2. Synthesis of β-amyloid inhibitors **4** and **5**.



Scheme 3. Synthesis of β-amyloid inhibitor **1**.

However, attempts to use this general approach for the coupling of diazonium salt **6** with aniline to produce the desired compound **5** were unsuccessful. Thus, aniline was protected with sodium formaldehydebisulfite,[16,17] followed by coupling with diazonium salt **6** to yield compound **4**. Alkaline hydrolysis of the protected amino derivative **4** with NaOH solution provided aniline derivative **5** in 73% yield (Scheme 2). While 4-[4-(2-diethylamino-ethylamino)-1-naphthylazo]-benzenesulfonamide[18] as our reference inhibitor **1** was synthesized according to procedures in the literature[19,20] (Scheme 3).

The proposed structural assignments were confirmed by detailed ^{1}H, ^{13}C NMR (HMQC, HMBC, COSY), and HR-EIMS analyses.

The $A\beta_{40}$ aggregation inhibitory activities of the synthetic compounds were measured using a thioflavin-T (ThT) fluorescence binding assay. As shown in Figure 3, the compounds were tested first at a high concentration (100 μM) and the relative ThT fluorescence, and hence, inhibition of $A\beta_{40}$ aggregation was determined. Results showed that compounds **1**–**5** significantly suppressed ThT fluorescence. In addition, the neurotoxicities were measured on cortical neurons using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay.[21–23] After incubation of cortical neurons

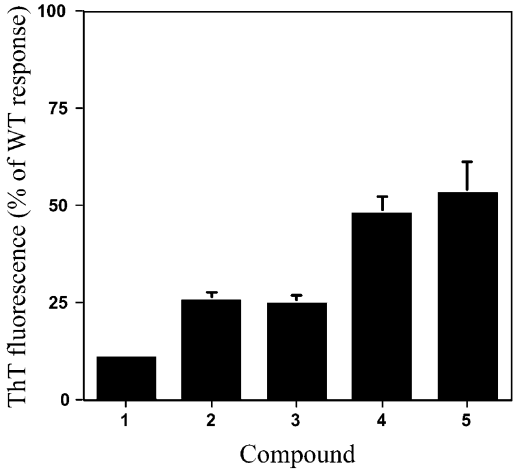


Figure 3. ThT fluorescence at Ex 450/Em 482 of $A\beta_{40}$ aggregation. The compounds (100 μM) and $A\beta_{40}$ (25 μM) were mixed in 50 mM TBS at pH 7.4 and incubated at 37 °C for 2 days, which promotes aggregation of $A\beta_{40}$. The figure shows the means ± S.D. ($n = 3$). All data were scaled with the wild type (WT) response of $A\beta_{40}$ alone (100% fluorescence).

Table 1. Effects of **1**–**5** on fibril formation and neurotoxicity[a]

Compd	R	EC_{50} (μM)[b]	IC_{50} (μM)[c]	TI (IC_{50}/EC_{50})[d]
1	4-$NH(CH_2)_2NEt_2$	15.11 ± 5.15	22.14 ± 3.01	1.47
2 (**LB-152**)	4-NMe_2	12.89 ± 0.83	> 100	> 7.75
3	2-SMe, 4-NH_2	56.79 ± 7.79	> 100	> 1.76
4	4-$NHCH_2SO_3Na$	77.23 ± 9.35	> 100	> 1.29
5	4-NH_2	> 100	> 100	no suppression

[a] All data are presented as average values from three separate experiments.
[b] Effect on fibril formation; concentration which inhibits wild type aggregation by 50%.
[c] Neurotoxicity; concentration which inhibits viability of neurons by 50%.
[d] Therapeutic index, $TI = IC_{50}/EC_{50}$.

with MTT at 37 °C for 1 h, the medium was removed and the formazan particles were dissolved with DMSO. $OD_{600\ nm}$ was measured by ELISA reader. The therapeutic index of these compounds was determined as follows. (1) $A\beta_{40}$ aggregation inhibitory activity was determined using ThT binding assay at various concentrations of compounds and the EC_{50} values were calculated. (2) Neurotoxicity was determined using the MTT reduction assay at various concentrations of compounds and the IC_{50} values were calculated. (3) The therapeutic activity was determined as therapeutic index (TI, IC_{50}/EC_{50}). The reference compound **1** was included during each bioassay for comparison.[11] Data are presented in Table 1.

As shown in Table 1, we successfully identified novel small-molecule inhibitors for inhibition of $A\beta_{40}$ aggregates. The present structure–activity relationships (SAR) demonstrate that modification on the end of the phenylazo moiety at the *para*-position with different amino substituents results in significant effects on the $A\beta_{40}$ aggregation inhibitory activity and neurotoxicity. Results of these SAR conclusions are discussed below.

(i) A simple phenylazo-like structure afforded good activity. (ii) None of the *trans*-stilbene benzenesulfonamide derivatives displayed $A\beta_{40}$ aggregation inhibitory activity (data not shown). (iii) Replacing the 4-(2-*N*,*N*-diethylamino-ethylamino)-naphthylamino moiety in **1** with a *p*-*N*,*N*-dimethylamino-phenylazo group in **2** (**LB-152**) did not significantly decrease activity, as reflected in the relative EC_{50} values of 15.11 and 12.89 μM, respectively. However, the bulkier naphthylazo **1** showed increased neurotoxicity with an IC_{50} of 22.14 ± 3.01 μM as compared to $IC_{50} > 100$ μM with phenylazo derivatives **2**–**5**. Consequently, **LB-152** had a TI value > 7.75, which is approximately 5.3-fold higher than the TI value of 1.47 for compound **1**. Additional mechanism studies are ongoing to better understand this result. (iv) Introducing the tertiary amino group showed the strongest $A\beta_{40}$ aggregation inhibitory activity compared to the primary or secondary amino group (**2** versus **3**–**5**). These results suggested that the relatively high lipophilicity of the *p*-*N*,*N*-dimethylamino group (an electron-donating substituent) on the end of the phenylazo moiety was very important for $A\beta_{40}$ aggregation inhibitory activity.

In summary, we have synthesized and evaluated a new series of *para*-substituted phenylazo benzenesulfonamides as potential $A\beta_{40}$ aggregation inhibitors. The *p*-*N*,*N*-dimethylamino substituted **LB-152** possessed the highest $A\beta_{40}$ aggregation inhibitory activity without inducing neurotoxicity.

Inhibition of $A\beta_{40}$ aggregation and reduction in neurotoxicity are expected to slow down or to arrest the progress of AD, as well as to prevent the earliest form of Aβ deposition. As mentioned earlier, one of the challenges in developing new inhibitors is to find nontoxic, non-peptide small-molecule lead compounds that can cross the BBB. Our studies have identified **LB-152** as a useful lead compound for future design of promising clinical trials candidates for prevention and/or retardation of amyloidogenesis involved in the development of AD. A more detailed analysis of the profile of **LB-152** as a potent β-amyloid aggregation inhibitor for treatment of AD will be presented in due course.

Acknowledgements

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