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Sulfonamides as multifunctional agents for Alzheimer's disease

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ABSTRACT

Sulfonamide linker-based inhibitors with extended linear structure were designed and synthesized with the aim of producing multifunctional agents against several processes involved in the pathology of Alzheimer's disease (AD). The potency of the compounds were assessed in the inhibition of Aβ self-assembly (fibril and oligomer formation), in modulating cholinesterase (AChE, BuChE) activity, and scavenging free radicals. Several compounds exhibited promising Aβ self-assembly and cholinesterase inhibition and in parallel, showed good free radical scavenging properties. The investigation of the scaffold described in this study resulted in the identification of three compounds (**14**, **19** and **26**) as promising leads for the further design of multifunctional drug candidates for AD.

Alzheimer's disease (AD) is a complex neurodegenerative disorder of the central nervous system.[1,2] In response to the pressing need of an aging population several treatment strategies have been explored.[3–7] The most common approaches are related to the cholinerg[8] and amyloid cascade hypotheses.[9] The formation of Aβ peptide self-assemblies (oligomers and fibrils) and their neurotoxic effects are believed to be major contributors to the development of AD.[10] Due to the potent effect of multiple Aβ neurotoxic products on disease development, a broad range of Aβ self-assembly inhibitors have been identified.[11] In addition, a low level of certain neurotransmitters, such as acetylcholine (ACh), is also associated with the disease.[12] The first generation of AD drugs were acetylcholinesterase (AChE) inhibitors with the goal of reducing ACh breakdown and therefore increasing ACh concentration, to provide symptomatic treatment.[13] In addition to AChE another cholinesterase enzyme, butyrylcholinesterase (BuChE), also appeared to negatively affect the level of neurotransmitters.[14] Recent studies indicate that the peripheral binding site of AChE may contribute to the initiation of Aβ self-assembly, as well.[15] In vivo studies also suggested elevated levels of both metals and oxidative stress are present in the AD affected brain.[16] There is a growing sentiment that multitarget therapeutics may combat the complex pathogenesis of the disease more effectively than single-target approaches.[17]

Extending our recent efforts on the development of anti-amyloidogenic compounds[18–24] we describe the synthesis, biochemical evaluation, and potential multifunctional application of sulfonamide-based small molecule agents, including saccharin, and other analogues. Sulfonamides exhibit a broad range of biological effects and are well-tolerated in biomedical applications. Their applications include use as early antibacterial agents[25] or saccharin, one of the most commonly sold artificial sweeteners.[26] Sulfonamides have also been found to be beneficial in AD. Kumar *et al.* proposed *N*-aryl sulfonamide substituted 3-morpholino arecoline derivatives for the symptomatic treatment of Alzheimer's dementia.[27] Other reports include the inhibition of amyloid-β formation from β-amyloid precursor protein (APP) or its self-assembly by a series of sulfonamide derivatives.[28–31] The sulfonamide analogs reported in this study are either novel and their synthesis never reported, or although commercially available their testing as multifunctional anti-AD agents has not been reported. We tested these compounds in a series of assays, including the inhibition of Aβ self-assembly, modulation of cholinesterase activity, and assessment of potential antioxidant properties.

The above reports inspired the investigation of several small commercially available sulfonamides (Fig. 1 and **1**–**6**).

The commercial compounds were evaluated in the inhibition Aβ self-assembly, including fibrillogenesis and oligomer formation inhibition, modulation of cholinesterase activity and free radical scavenging. Some compounds showed promising results in these assays and also pointed toward possible improvements. We decided to synthesize additional derivatives focusing on the extension of the length of the compounds.

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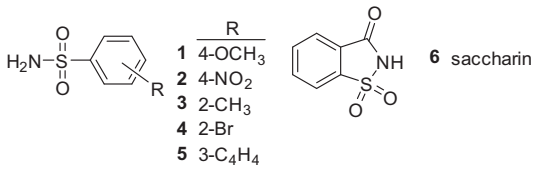


Figure 1. Structure of the tested commercial sulfonamides.

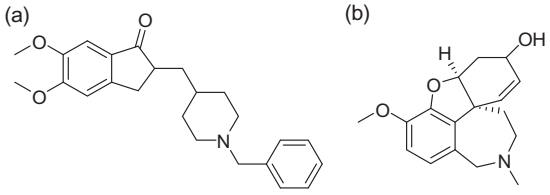


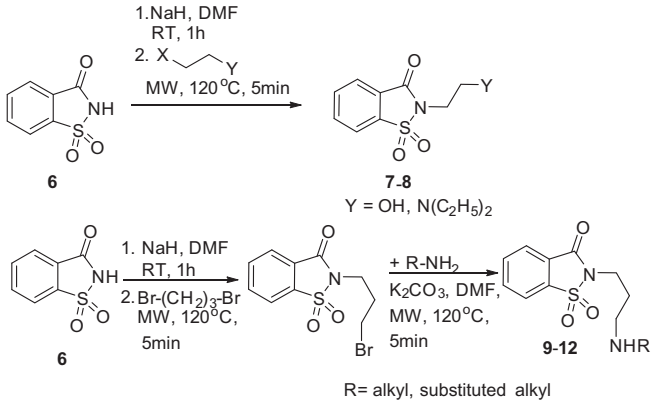
Figure 2. Structure of donepezil (a) and galanthamine (b).

We expected that compounds with longer alkyl chains might act as better cholinesterase inhibitors, mimicking the structural features of donepezil (Fig. 2a); a well-known AChE inhibitor without decreasing the efficiency of the compounds in other assays.[32] Therefore, several saccharin derivatives (**7**–**12**) were synthesized as illustrated in Scheme 1.

After the preliminary assays further modifications were executed on the scaffold. Instead of having the sulfonamide moiety in a ring, a relatively long, linear chain was introduced to the scaffold, positioning the sulfonamide group in the chain. Aromatic head and tail groups were added as well. This design aims to provide more flexibility to the compounds and at the same time to continue to have large flat end-units. The synthesis of these second generation compounds (**14**–**26**) is summarized in Scheme 2a.

Two additional compounds; **27** and **28** were also prepared (Scheme 2b) to determine how the activity would be affected if the sulfonamide moiety was eliminated from the scaffold. Each product was characterized by ^{1}H and ^{13}C NMR spectroscopy and by LC–MS. The spectroscopic characterization of the new compounds was in agreement with their structures (see Supporting information). The complete list of the compounds synthesized in this work, except **27** and **28** (Scheme 2b), is shown in Figure 3.

To determine the activity profile of the compounds they were first subjected to Aβ fibrillogenesis assays. The quantitative Thioflavin-T (THT) fluorescence assay was applied to determine the antifibrillogenic potency of the compounds.[33] All data were normalized to the fluorescence of the inhibitor-free control ($I_{control}$). The fibrillogenesis assay data are presented in Figure 4.



Scheme 1. Structure of the tested commercial sulfonamides.

The data indicate that the compounds overall belong to two different groups. The commercially available sulfonamides (**1**–**6**) and the rather compact saccharin derivatives (**7**–**12**) commonly promote fibril formation. In contrast, the long linear-shaped compounds (**13**–**28**) exhibited moderate to good fibrillogenesis inhibition. Compounds **14**, **18**, **19**, **21** and **26** showed the most significant effect (87%, 60%, 43%, 64% and 45%, respectively). To confirm that inhibition was not due to THT displacement by compounds during the above assays, complementary Atomic Force Microscopy (AFM) experiments were performed.[34] For example, AFM images of the solvent treated control and the sample incubated in the presence of compound **14** (Fig. 3), which showed the best (87%) fibril inhibition are depicted in Figure 5.

The images confirm the interpretation of the THT results. The control sample showed the expected well-developed mature fibrils, while the image of the inhibitor-containing sample reflects the high, although not complete, inhibition. The small amount of remaining fibril-like assemblies were thinner and shorter in appearance indicating the profound effect of **14** on the Aβ fibrillogenesis.

The compounds were also tested for their activity in the inhibition of oligomer formation by the biotinyl-Aβ(1–42) single-site streptavidin-based assay.[35] The samples were incubated for 30 min in the assays, using Aβ/inhibitor = 0.0002 ratio at 0.01 μM Aβ concentration. The intensity of the inhibited samples was normalized to the control sample containing Aβ and the solvent. The percentile values were calculated similarly to those of fibril inhibition as shown above. The data are illustrated in Figure 6.

Comparing Figure 6 to Figure 4, indicates that compounds active against oligomers were poorly active against fibrillogenesis and vice versa. The small commercial sulfonamides and saccharin derivatives (**1**–**12**) showed moderate inhibition (up to 54%, except **11**) of oligomers while the long chain linker containing sulfonamides (**13**–**26**) were generally oligomer formation promoters. Interestingly, **27** and **28** showed 50–100% inhibition of oligomer formation. These compounds are similar to the long chain sulfonamides (**13**–**26**), however, they lack the sulfonamide moiety. Comparing the activity of the compounds in the two Aβ self-assembly inhibition assays one cannot fail to notice that the behaviour of the compounds in the two assays are the opposite; a compound is either a fibril inhibitor or an oligomer inhibitor. This observation is in agreement with our earlier findings[21–24] and literature data.[10]

Oxidative stress caused by free radicals also plays an important role in development of AD. Thus, the potential antioxidant character of the compounds was also assessed. The scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was measured by the decrease of its absorbance The data are compared to those obtained with reference compounds ascorbic acid[36] and resveratrol,[37] both of which are well-known antioxidants. The data are illustrated in Figure 7.

Three of our synthetic sulfonamides (**19**, **23** and **24**) showed higher free radical scavenging property than ascorbic acid, two molecules (**23** and **24**) were even better than resveratrol.

With an aim to have multi-target functionality each compound was assayed for the inhibition of cholinesterases. All of the synthesized molecules were subjected to Ellman assay of the hydrolysis of acetylthiocholine to determine their potency towards inhibition of AChE and BuChE (Figs. 8 and 9). For AChE inhibition, the molecules were assayed at 2 M; which is the IC_{50} of galanthamine (Fig. 2b).[38] Molecule **19** (Fig. 3) produced 88% inhibition at 2 μM, while galanthamine at same concentration showed 54% inhibition. Molecules **18** and **26** (Fig. 3) showed greater than 40% inhibition at the same concentration. Due to solubility issues molecule **28** (Scheme 2) could not be tested in either assay. For BuChE inhibition, the molecules were assayed at 10 μM; which is the IC_{50} of galanthamine against that enzyme.[39] Nine out of 15 synthesized