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Design, Synthesis and Biological Activity of Multifunctional α,β-Unsaturated Carbonyl Scaffolds for Alzheimer's Disease

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Abstract

A series of compounds containing an α,β-unsaturated carbonyl moiety, such as chalcones and coumarins were designed, synthesized and tested in a variety of assays to assess their potential as anti-Alzheimers' disease (AD) agents. The investigations included the inhibition of cholinesterases (AChE, BuChE), the inhibition of amyloid beta (Aβ) self-assembly and the disassembly of preformed Aβ oligomers. Several compounds showed excellent inhibition in multiple assays and thus are potential multifunctional compounds for AD. Docking studies for **16** that performed well in all the assays gave a clear interpretation of various interactions in the gorge of AChE. Based on the results, the long-chain coumarin scaffold appears to be a promising structural template for further AD drug development.

Alzheimer's disease (AD) is associated with a loss of presynaptic markers of the cholinergic system in the areas of the brain related to memory and learning, and is also characterized by the presence of amyloid deposits and neurofibrillary tangles in the brain.[1] The deterioration of cholinergic neurotransmission is responsible for the decline in memory and cognition in patients suffering from AD.[2] One of the ways to enhance cholinergic function by preserving acetylcholine (ACh) levels is to inhibit acetylcholinesterase (AChE) responsible for the metabolic breakdown of ACh. Preclinical experiments and clinical trials have shown butyrylcholinesterase (BuChE) to be an important contributor for the occurrence, symptoms, progression, and responses to treatment in dementia.[3,4] Several anti-cholinergic drugs have been launched including tacrine, rivastigmine, donepezil, and galanthamine.[5] It has also been shown that dual AChE/BuChE agents might be superior to the selective AChE inhibitors.[2]

AChE has a long and narrow gorge (20 Å) with two binding sites; a catalytic site (active site) and a peripheral anionic site (PAS).[6] It was reported that the peripheral site of AChE could promote the formation of amyloid beta (Aβ) deposits.[7,8] The formation of such deposits is a hallmark of AD pathology.[9,10] Detailed studies[11] indicated that both fibrillar and oligomeric species of Aβ are neurotoxic.[12,13] Thus the design of multifunctional

inhibitors, which can control the hydrolytic activity of cholinesterases and interfere with the self-assembly of Aβ and disassemble the preformed aggregates is highly desirable.[14, 15, 16]

Continuing our earlier work on novel anti-AD compounds,[17,18,19,20,21] we describe novel compounds with an α,β-unsaturated carbonyl moiety (chalcones and coumarins) that can act as multifunctional anti-cholinesterase/Aβ agents. Chalcone[22] and coumarin[23] derivatives have been described as AChE and BuChE inhibitors. While coumarins have also been applied as Aβ aggregation inhibitors,[24] chalcones are more commonly used as Aβ imaging agents.[25,26] There are no reports that such compounds synthesized or applied as multifunctional agents such as inhibiting both cholinesterases as well as Aβ self-assembly.

We designed α,β-unsaturated carbonyl compounds with an open chain (chalcones) and cyclic (coumarins) structures. In order to have dual binding property in the gorge of AChE the α,β-unsaturated carbonyl unit was linked to different amines. The designed structures were then screened through the 4-point pharmacophore developed in our group[27] and molecules that matched 3 of the 4 points of our pharmacophore (Fig. 1) were synthesized.

The syntheses of the compounds are summarized in Schemes 1 and 2.[28] The synthesis of the target compounds applied readily available starting materials and was completed using well established methods.[25]

The compounds were tested by the Ellman colorimetric assay for cholinesterase inhibition.[29,30,31] For comparison, galanthamine (**GAL**), a known cholinesterase inhibitor, was used. The experiments were carried out with 2 μM and 10 μM inhibitor concentration, respectively (IC_{50} of **GAL** for AChE and BuChE) (Fig. 2).[6,7] **GAL** was selected due to its low toxicity and ready commercial availability.

The data (Fig. 2) indicate that the compounds possess appreciable dual cholinesterase inhibition. Coumarins are potent AChE inhibitors while chalcones show stronger activity in BuChE inhibition. In AChE inhibition most inhibitors showed comparable but somewhat weaker inhibition than that of the reference compound (**GAL**). Compound **16** exhibited higher activity than **GAL** and showed excellent (57%) inhibition at 2 μM (IC_{50} of **GAL**) with an IC_{50} of 1.76 μM.

The compounds behaved similarly in BuChE inhibition assays. Chalcones possess activity similar to **GAL** and a few compounds showed more than 50 % inhibition at 10 μM (IC_{50} of **GAL**), however, most of their IC_{50} values were determined to be slightly higher than that of **GAL** except **15** with an IC_{50} of 8.27 μM. While the initial data suggested **11** being a slightly better inhibitor than GAL, the IC_{50} determination did not confirm this likely due to the deviation of the data.

In order to understand the interactions of **16** with the enzyme, it was docked in the active site of AChE (PDB code: 1E66) (Fig. 3) using SP mode of glide module of Schrödinger.[32] For comparison compound **16** was superimposed with galanthamine and another well-known AChE inhibitor, donepezil (Fig. 3). Unlike galanthamine, **16** spanned the active site and peripheral anionic site of the enzyme similarly to donepezil. As shown, **16** is oriented properly in the active site (Fig. 3). The coumarin ring was stabilized by hydrophobic interactions with Trp 333, Phe 290 and Phe 288 residues. The phenyl ring at the amino end was stabilized by hydrophobic and π-π interactions through Trp 84 and Trp 130. The methoxy group of coumarin ring formed a hydrogen bond (Phe 288) and the –NH of the piperazine ring formed a hydrogen bond with Ser 122. These observations explain the potency of **16** in AChE inhibition.

As fibrillar and oligomeric aggregates of Aβ are neurotoxic, the activity of the compounds was determined against the formation of these species and in the disassembly of preformed oligomers. The compounds were tested by standard methods, the biotinyl-Aβ(1-42) single-site streptavidin assay for oligomer formation and disassembly[33,34] (Fig. 4) and the thioflavine T-fluorescence assay for fibril formation (Fig. 5).[35,36,37]

In the oligomer assembly assay coumarins (**16-22**) are excellent inhibitors (> 65%, Fig. 4A) while chalcones showed little or no inhibition (Fig. 4A). The concentration dependence studies revealed the following IC_{50} values: **16** (36 μM), **17** (14 μM), **18** (21 μM), **20** (1 μM), **21** (29 μM), **22** (36 μM).

The same group of compounds (**16-22**) effectively (up to 95%) disassembled the preformed oligomers as well (Fig. 4B). The IC_{50} data for oligomer disassembly are: **17** (29μM), **18** (6.5 μM), **20** (4 μM) and **21** (5 μM). The compounds exhibited similar behavior in the inhibition of Aβ fibril formation (Fig. 5). Both chalcones (**1-15**) coumarins (**16-22**) were reasonable fibrillogenesis inhibitors (~ 30-70%). It is worth noting that the compounds did not show significant intrinsic fluorescence thus their presence in the assay did not affect the fluorescence readings.

Atomic force microscopy (AFM) was used to confirm the fibrillogenesis inhibition (Fig. 6). The images revealed the expected dense network of long fibrils in the control sample. While fibrils are still visible in the inhibited samples the density is significantly lower in agreement with the assay results. It also can be observed that shorter assemblies formed in the presence of **16**.

Chalcones and coumarins have already been investigated to modulate cholinesterase activity or inhibit Aβ self-assembly, respectively.[22-26] In an attempt to combine these activities in a single chemical structure and develop multifunctional anti-AD compounds we synthesized 22 compounds with chalcone and coumarin head and varied tail groups. The aim was to build compounds of sufficient length to span the active center of the cholinesterases and at the same time interfere with Aβ self-assembly. The structure-activity relationship reveals important information for future design. It appears that the above results obtained with coumarins support the hypothesis. Chalcones showed strong activity in BuChE and fibrillogenesis inhibition only. They were relatively weak inhibitors of AChE and Aβ oligomer formation. In contrast, coumarins performed more consistently showing significant inhibition in all assays, except against BuChE where they were moderate to weak inhibitors. At this point the coumarin derivatives were limited to one head group and different tail groups possessing aryl-substituted piperidine, piperazine and morpholine units. Compounds with the two nitrogen containing piperazine ring (**16**, **18-20**) performed the best overall. While the observed differences in activity within this subgroup are not decisive, the data suggest that the most beneficial tail groups are phenyl and benzyl piperazines (**16**, **18**). It appears that the presence of basic nitrogens in the middle ring is advantageous; however, too many nitrogens (e.g. pyrimidinyl-piperazine group, **20**), result in a slight decrease in activity in all assays.

Comparing the anti-oligomer and anti-fibril assays one may notice that while coumarins almost completely inhibit the formation of oligomers the same compounds are moderate inhibitors (~65%) of the fibril formation. It is most likely due to the fact that there are multiple pathways involved in the amyloid formation and the oligomers are not obligatory precursors to the fibrils.[38]

In conclusion, a variety of compounds with α,β- unsaturated moiety were synthesized with the aim of preparing multifunctional compounds with anticholinesterase and anti-Aβ

assembly activities for AD. It was observed that while the open chain derivatives (chalcones) were inactive in two important assays the cyclic coumarins showed excellent to reasonable effects in several assays, e.g. cholinesterase activity and Aβ self-assembly. Based on the structure-activity relationship, compounds with a coumarin-based head group and an aryl/benzyl-piperazine tail group are promising leads for further inhibitor design. The structures of our lead compounds represent a relatively nonpolar character with an extended network of hetero- and carbocyclic rings. This feature suggests favorable membrane permeability, which is an important factor for drug candidates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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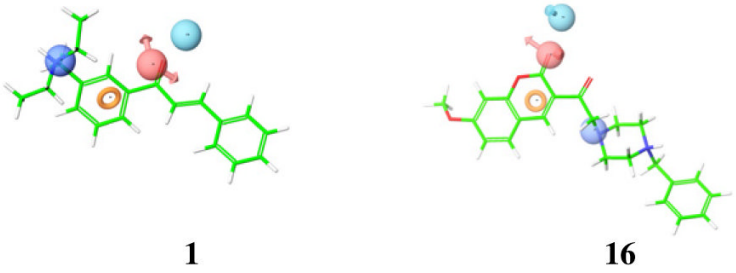


Figure 1.
Pharmacophore features of two of the designed compounds. Light blue sphere-H-acceptor; red sphere–H-donor; ring – aromatic ring and dark blue sphere – positively charged center

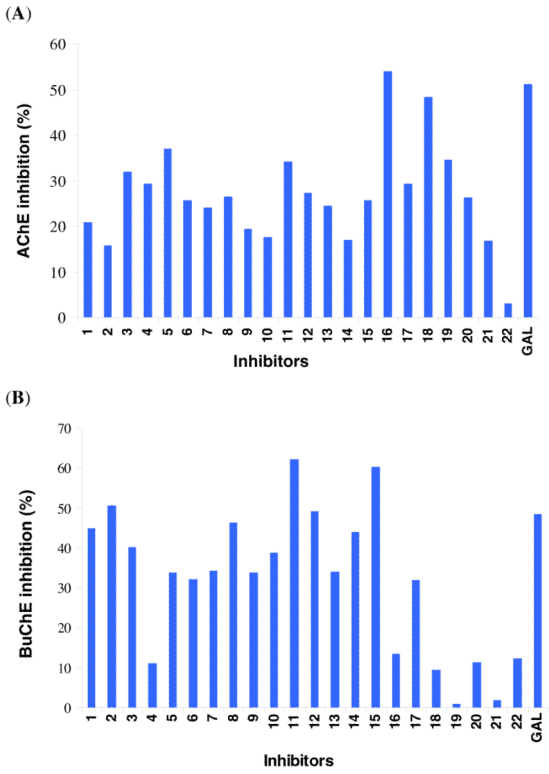


Figure 2.

Inhibition of (**A**) AChE and (**B**) BuChE hydrolytic activity by chalcones and coumarins. The experiments were carried out at 2 μM (**A**) and 10 μM (**B**) inhibitor concentration and 0.02 unit/mL enzyme concentration.[29]

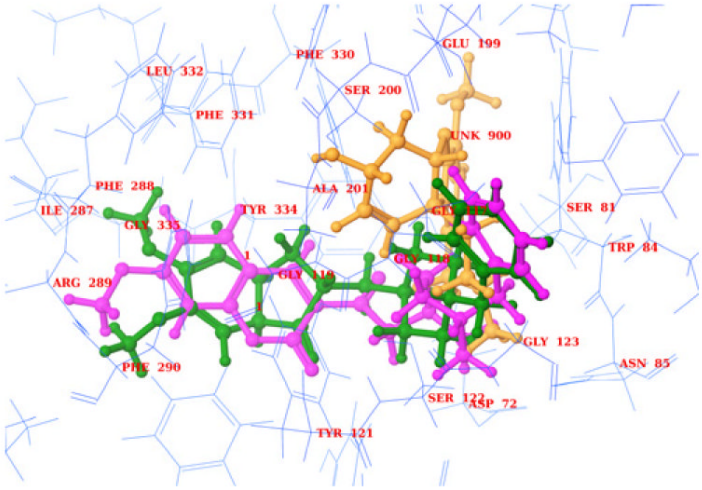


Figure 3.
Superimposition of **16** (pink, ball and stick) with donezepil (green, ball and stick) and galanthamine (brown, ball and stick) in the active site of 1E66.

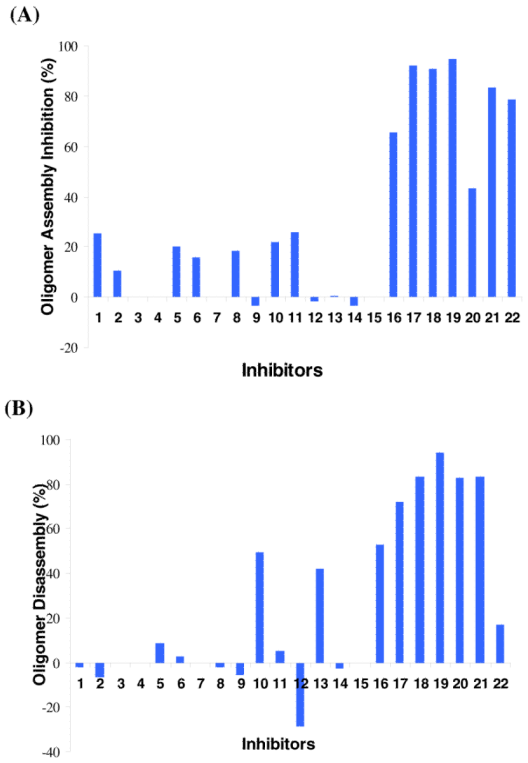


Figure 4.
Effect of the designed compounds on the (A) oligomer assembly of Aβ and (B) disassembly of preformed Aβ oligomers (50μM inhibitor; 0.01μM Aβ-inhibition; 0.0028μM Aβ-dissociation)

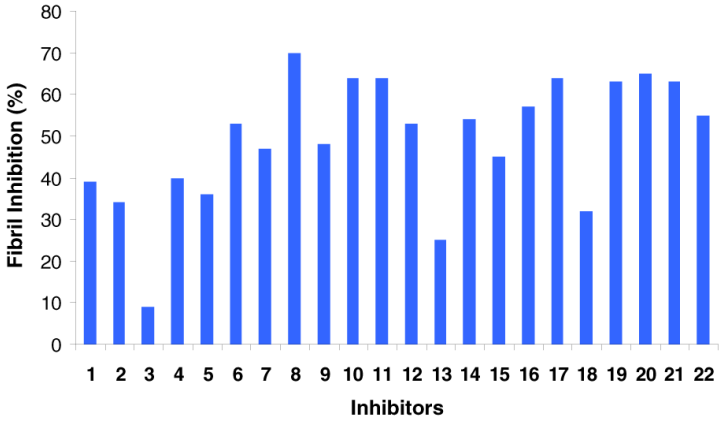


Figure 5.
Effect of the designed compounds on the fibrillogenesis of Aβ (100 μM inhibitor, 100 μM Aβ)

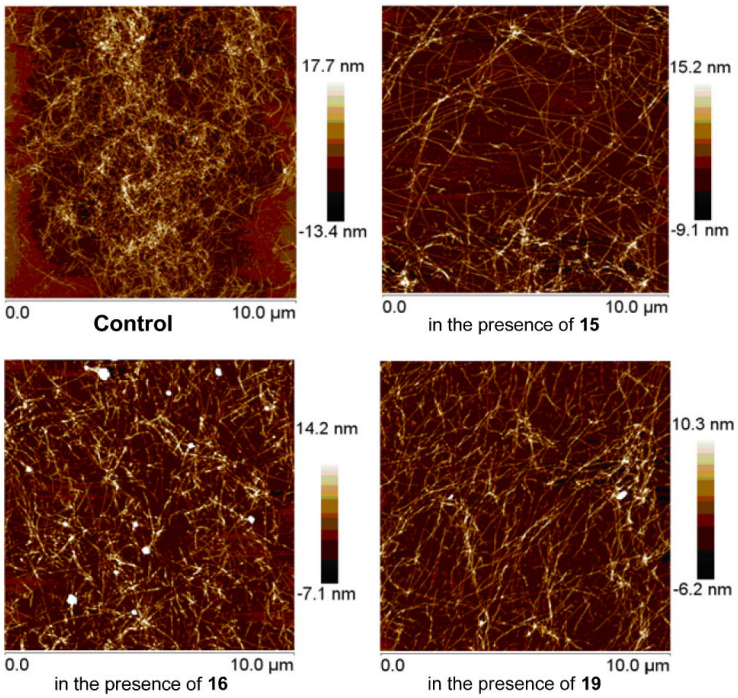
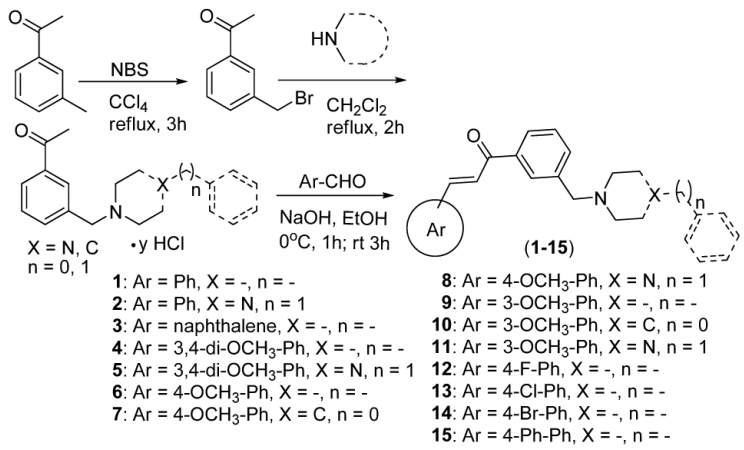
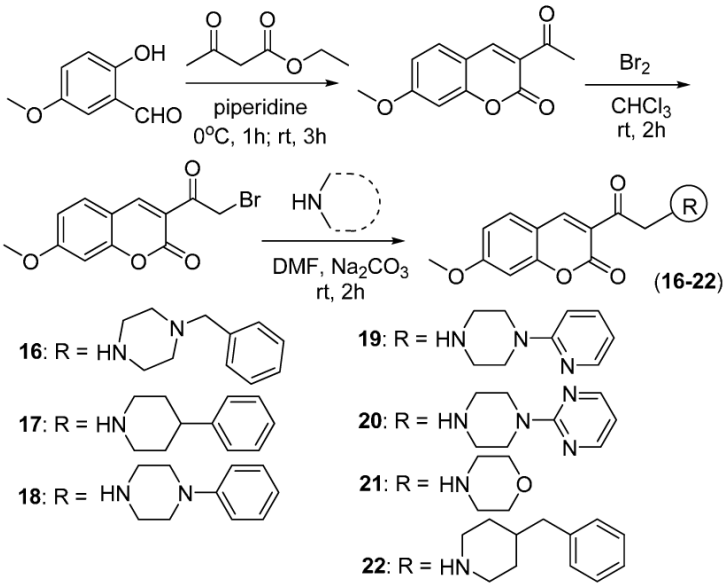


Figure 6.
Illustrative images of the control and selected samples in the presence of compounds **15**, **16** and **19**, respectively.



Scheme 1.
Synthesis and structures of chalcone derivatives used



Scheme 2.
Synthesis and structures of coumarin derivatives used