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Discovery of Notch-Sparing γ -Secretase Inhibitors

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Abstract

Overwhelming evidence supports a central role for the amyloid β -peptide ($A\beta$) in the pathogenesis of Alzheimer's disease (AD), and the proteases that produce $A\beta$ from its precursor protein APP are top targets for therapeutic intervention. Considerable effort has focused on targeting γ -secretase, which generates the C-terminus of $A\beta$; however, γ -secretase inhibitors cause serious toxicities due to interference with the Notch signaling pathway. We have been working toward compounds that directly alter γ -secretase activity to reduce $A\beta$ production without affecting the proteolysis of Notch. Using purified enzyme and substrate, we have shown that γ -secretase can be selectively inhibited in this way by naphthyl-substituted γ -aminoketones and γ -aminoalcohols. These early hits, however, suffered from chemical instability and/or poor potency. Iterative design, synthesis and evaluation have led to the discovery of Notch-sparing γ -secretase inhibitors with substantially increased potencies in biochemical and cellular assays. These compounds are of low molecular weight and are under evaluation for drug-like properties. The discovery and development of these compounds will be discussed.

γ -Secretase catalyzes proteolysis of the transmembrane region of the amyloid β -protein precursor (APP) to generate the amyloid β -protein ($A\beta$) and is a top target for the development of disease-modifying therapeutics for Alzheimer's disease. This protease is a complex of four different integral membrane proteins: presenilin, nicastrin, Aph-1, and Pen-2 [1]. Presenilin contains two completely conserved transmembrane aspartates that are essential for γ -secretase activity, part of the compelling evidence that presenilin is a novel, membrane-embedded aspartyl protease. Although presenilin is the catalytic component of γ -secretase, it nevertheless requires the other three components to become an active protease and to maintain activity.

Many highly potent inhibitors of γ -secretase that readily penetrate biological membranes have been identified. However, these compounds interfere with the processing of other substrates of this protease in addition to APP [2] which raises serious concerns about selectivity and toxicity. γ -Secretase can cleave a number of different single-pass membrane proteins. However, the most pharmacologically relevant alternative substrate is the Notch receptor. Signalling from this receptor plays a role in many cell differentiation events that occur from embryogenesis into late adulthood.

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Competing interests

The authors claim no competing interests.

Authors' contributions

This article was written by MSW and CEA. Compound synthesis was carried out by HW, DL and JZ. Compound evaluation was carried out by YG and TY.

The Notch signal is initiated by interaction with a cognate ligand that induces shedding of the extracellular portion of the receptor. The remaining membrane-bound stub is then processed by γ -secretase to release an intracellular domain that translocates to the nucleus and directly interacts with certain transcription factors, thereby regulating gene expression. Because γ -secretase is essential for Notch signaling, inhibitors of this protease can interfere with cell differentiation. For example, treatment of mice with γ -secretase inhibitors over time can cause severe gastrointestinal toxicity and compromise the proper maturation of B- and T-lymphocytes [3,4]. Thus, selective inhibition of γ -secretase-mediated cleavage of APP without affecting the proteolysis of Notch is a major goal toward realizing practical therapeutics for AD.

Two types of compounds appear to selectively modulate γ -secretase activity via direct interaction with the protease or its substrate. The first of these are a subset of non-steroidal anti-inflammatory drugs (NSAIDs) that shifts the production of A β away from the more aggregation-prone 42-residue variant (A β 42) and towards a shorter, more soluble 38-residue variant (A β 38) [5]. These compounds include ibuprofen, indomethacin, and sulindac sulfide. The effects of these compounds were demonstrated in isolated membranes [6], suggesting that the compounds work directly on enzyme or substrate instead of indirectly via a signaling or metabolic pathway [7]. On the other hand, evidence supports the APP substrate itself, specifically its juxtamembrane region, as the direct binding site, which would explain the putative selectivity of these compounds for APP versus Notch [8]. One of these compounds, R-flurbiprofen (tarenflurbil), failed in late-stage clinical trials for the treatment of AD due to its lack of efficacy, emphasizing the need for a better understanding of the mechanism and the structure-activity relationships of this class of compounds towards improving potency and selectivity.

Certain kinase inhibitors can also selectively affect A β production at the γ -secretase level with little or no effect on Notch proteolysis. Because ATP was found to augment the γ -secretase cleavage of C99 to A β , the Greengard laboratory at Rockefeller University tested kinase inhibitors (i.e., compounds that interact with ATP binding sites) for their ability to prevent A β production. The Abl kinase inhibitor imatinib (GleevecTM) was found to block A β formation without affecting Notch [9]. This action of imatinib was not due to an interaction with Abl kinase, although the assumption was that some membrane-associated kinase was the target. Subsequently, our laboratory found that an extract from the drug's capsules (but not imatinib itself) could inhibit A β production from purified γ -secretase while leaving the proteolysis of Notch unaffected [10]. We also found that an inhibitor of Janus kinase 3 (Jak3) showed selective inhibition on purified γ -secretase (compound 1367; Fig. 1; IC₅₀ = 20 μ M). Further experiments revealed a nucleotide binding site on the γ -secretase complex. For example, affinity-labelling with a photo-reactive azido-substituted ATP led to its covalent attachment to PS1. This labelling was prevented by the imatinib extract and the Jak 3 inhibitor, but not by a transition-state analogue inhibitor (i.e., directed to the active site). These findings suggested a specific competition with ATP for binding to the γ -secretase complex at an allosteric site [10].

Our original hit, compound 1367, is a naphthyl-containing aminoketone that undergoes elimination of the amine portion at neutral pH to produce the vinylketone 1366 (Fig. 1). This compound also displayed selective inhibition of γ -secretase cleavage of APP vis-à-vis Notch in the purified enzyme assay (IC₅₀ = 60 μ M) [10], which raised the question of whether the activity of 1367 is solely due to its degradation to 1366 or if 1367 had any intrinsic activity of its own. Toward this end, we designed and synthesized the alcohol form of 1367 (dubbed compound AD29; Fig. 1). This compound is chemically stable: The amino group is not capable of elimination because there is no ketone functionality and therefore no labile α -proton. Testing in our purified γ -secretase assays revealed that AD29 retained selective

inhibition of A β production with an IC₅₀ of 40 μ M. When tested in cells, AD29 inhibited APP processing with an IC₅₀ of 88 μ M. In other attempts to stabilize compound 1367, the distance between the carbonyl functionality and the amino atom was varied to separate the ketone from the amino group. When the length of the alkyl chain between the carbonyl and the amide was longer than that of AD29, potency was diminished. However, when this alkyl chain was completely removed, resulting in amide analogue AD33 (Fig. 2), selective inhibition of γ -secretase production of A β was retained.

In light of these findings, a variety of analogues of AD29 and AD33 were synthesized. However, while many of these analogues retained selective inhibition of γ -secretase cleavage of APP compared to Notch, the potencies remained in the mid-micromolar range. This plateau of potency was finally broken when the amide functionality of AD33 was replaced with a sulfonamide (i.e., swapping atoms circled as B in Fig. 2 from carbonyl or sulfonyl). Within a few months time, over 180 sulfonamides of this general type were synthesized and tested, varying regions A and C in Fig. 2. Several of these newly synthesized sulfonamides selectively inhibit A β production from purified γ -secretase with IC₅₀ values ranging from 2 μ M down to 0.23 μ M. Furthermore, these same compounds inhibit A β production in cells with IC₅₀ values from 300 nM to as low as 27 nM and have not shown any cytotoxicity. Promising physicochemical properties (e.g., size, lipophilicity, polar surface area) suggest that these compounds warrant pharmacokinetic characterization, which in turn should indicate whether the compounds warrant further testing for their ability to lower A β in an APP transgenic mouse model.

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Fig. 1.
Hits 1367 and 1366 and stable lead compound AD29.

Fig. 2.
Arylamide AD33 (I) and sulfonamide analogues (II).