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Conversion of the LXR-agonist TO-901317—From inverse to normal modulation of γ-secretase by addition of a carboxylic acid and a lipophilic anchor

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Abstract—TO-901317, a LXR agonist, is an inverse modulator of Alzheimer's disease associated γ-secretase. We synthesized **TO-901317** analogous compound but replaced the hexafluorocarbinol moiety by an oxyacetic acid functionality and hypothesized that the replacement would change the mode of action from an inverse modulation to normal modulation of γ-secretase. As anticipated, acid **9** was found to be an effective modulator of γ-secretase and displayed activity at low micromolar concentration. This significant modification can be applied to several inverse γ-secretase modulators. Such modulators may preserve the cleavage of other γ-secretase substrates such as Notch.

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disorder characterized by memory loss, personality changes and a decline in cognitive abilities. An estimated 37 million people worldwide currently have dementia; AD affects about 18 million of them.[1] The pathological hallmarks of AD are extracellular amyloid plaques mainly composed of amyloid β-peptides (Aβ) and intracellular neurofibrillary tangles.[2] Aβ is generated by the sequential cleavage of β-amyloid precursor protein (APP) by β- and γ-secretases. APP is a type I transmembrane glycoprotein and exerts a role in neuro-protection, synaptic transmission, signal transduction and axonal transport.[3,4] APP is processed by β-secretase to produce soluble APPβ and the membrane-bound C-terminal fragment C99.[5] γ-Secretase cleaves the fragment C99 within its transmembrane domain to produce Aβ. $Aβ_{42}$ is more hydrophobic and believed to trigger the neuropathological cascade which ultimately leads to neurodegeneration.[6,7] The γ-secretase complex is an unusual transmembrane aspartyl protease which carries out the proteolytic cleavage within the lipid bilayer.[8] γ-Secretase occurs as a high molecular weight complex and is composed of at least four proteins: presenilin 1 (PS1), nicastrin, anterior pharynx defective-1 and presenilin enhancer-2. These four components form the active core of the γ-secretase complex[9] and PS1 provides the proteolytic site.[10–13] The γ-secretase complex processes several other type I transmembrane proteins such as Notch, E-cadherin, N-cadherin, CD44, DCC, ErbB4, LRP and nectin-1, which are involved in many physiological and pathological functions.[14] Purified γ-secretase complexes revealed that the membrane protein CD147 has a regulatory effect on γ-secretase activity.[15]

The mechanism of γ-secretase-mediated proteolysis is of great interest because it executes the final catalytic step in the APP processing which ultimately results in Aβ secretion. γ-Secretase activity can be controlled by the inhibition of PS1 which contains the catalytic subunit of the γ-secretase complex. PS1 harbours two aspartates in the transmembrane domains 6 and 7 that compose the active site.[16] Most of the reported and confirmed γ-secretase inhibitors are not substrate specific and interfere with Notch processing and signalling.[17,18] However, compounds capable of modulating the active γ-secretase complex by allosteric binding and subsequent interference with complex assembly or substrate recognition can alter or block Aβ production with little or no effect

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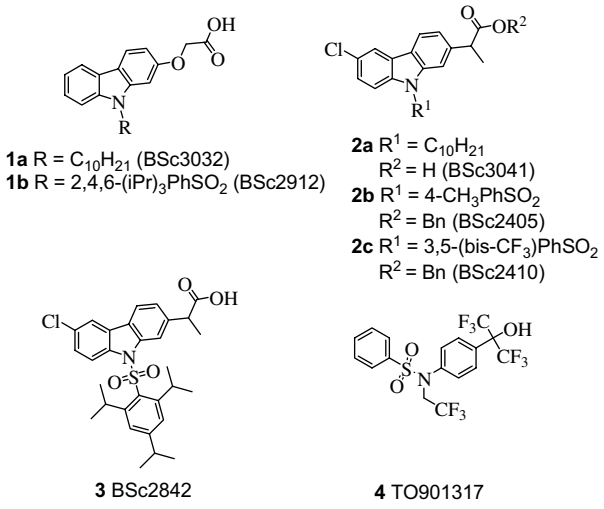
on Notch cleavage. Such compounds will be suitable candidates for AD therapeutics as they retain the cleavage of other γ-secretase substrates.[19,20] Certain nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g., ibuprofen, indomethacin, and sulindac sulfide) can reduce the production of the highly aggregation prone $A\beta_{42}$ peptide, and increase the level of $A\beta_{38}$ peptide, independent of their COX inhibitory activity.[21–23] Enzyme kinetics indicated that they might be interacting with a site distinct from the active site.[24] Recently, we reported that N-sulfonylated and N-alkylated carprofen and carbazolyloxyacetic acid derivatives modulate γ-secretase independent of their COX inhibition.[25,26]

The most active compounds **2** and **3** affected the cleavage at the γ38, γ40 and γ42 sites to a different extent and particularly suppressed the formation of $A\beta_{42}$. They enhanced the formation of $A\beta_{38}$ and thus showed the typical profile of effective NSAIDs. $A\beta_{38}$ lacks the four C-terminal hydrophobic amino acids of $A\beta_{42}$ and is far more soluble and less prone to aggregate; it has no known physiological function. Modification of the acid moiety into amides or esters resulted in the loss or inversion of modulatory activity (Table 1, compounds **2b** and **2c**); several NSAID derived esters turned out to be inhibitors of Aβ secretion.[25,26,29] This observation indicated an important contribution of the carboxylic acid to target affinity. Riddell et al. reported recently that **TO-901317**, a liver X receptor (LXR) agonist, reduced $A\beta_{42}$ levels in the Tg2576 mouse model at brain concentrations of 5 μmol/l and reversed the contextual memory deficit in these mice.[27] Due to the rather nonphysiological concentration they concluded that **TO-901317** does not directly interfere with APP processing and tentatively attributed the activity to an APOE-mediated mechanism. However, an opposite effect was observed in vitro: Czech et al. demonstrated recently that **TO-901317** interacts with γ-secretase at relevant physiological concentrations in a cell free assay to alter the production of different Aβ peptides.[28] Moreover, **TO-901317** directly modulates APP cleavage by γ-secretase in cellular assays and displays a cholesterol-independent effect on APP processing. It was found to be an inverse modulator of γ-secretase and displayed activity at the physiological concentrations required for LXR agonism. It selectively increased the formation of $A\beta_{42}$ and reduced the formation of $A\beta_{38}$.

We investigated the LXR agonist **TO-901317**, because it shares features of the structure–activity relationship for normal and inverse γ-secretase modulation observed for NSAIDs and curcumins,[25,26,29] but replaced the hexafluorocarbinol moiety by an oxyacetic acid functionality. We hypothesized that in analogy to NSAID derivatives the replacement of the hexafluorocarbinol moiety by an oxyacetic acid functionality would switch the mode of action from inverse to normal modulation.

Therefore, we synthesized the LXR agonist **TO-901317** analogous compound **9** as depicted in Scheme 1 and compared it to the structurally related NSAID derivatives **1**–**3**. Alkylation of the phenolic –OH of **5** using anhydrous K_2CO_3 and *tert*-butyl chloroacetate in acetone at 60–70 °C and subsequent nitro group reduction by hydrogenation using 10% Pd–C in ethyl acetate gave amine **6**. N-Sulfonylation of amine **6** was carried out using triethyl amine, catalytic DMAP and 3,5-bis-(trifluoromethyl) benzene sulfonyl chloride in DCM to afford



Scheme 1. Structures of the γ-secretase modulators and **TO-901317**.

Table 1. Activity report of compounds **1**–**9**

Entry	Compound	Compound code	Cell viability[a] (μM)	Activity in μM				
				$IC_{50}A\beta_{38}$	$EC_{50}A\beta_{38}$	$IC_{50}A\beta_{40}$	$IC_{50}A\beta_{42}$	$EC_{50}A\beta_{42}$
1	**1a**	**BSc3032**	>40		nd	>40	11	
2	**1b**	**BSc2912**	20		7.8	19	7.5	
3	**2a**	**BSc3041**	40		5.8	>40	2.9	
4	**2b**	**BSc2405**	>40	20 ± 5		na		20 ± 5
5	**2c**	**BSc2410**	>40	>20		>40		>20
6	**3**	**BSc2842**	40		9.3	>20	8.5	
7	**4**	**TO901317**	—	4.5		>40		3.6
8	**7**	**BSc3850**	>40	>40		>40		10.6
9	**8**	**BSc3851**	>40	13.3		>40		6.2
10	**9**	**BSc3769**	40		23.7	>40	19.6	

[a] Significant cellular toxicity was observed at this concentration. Viability reduction >20%.

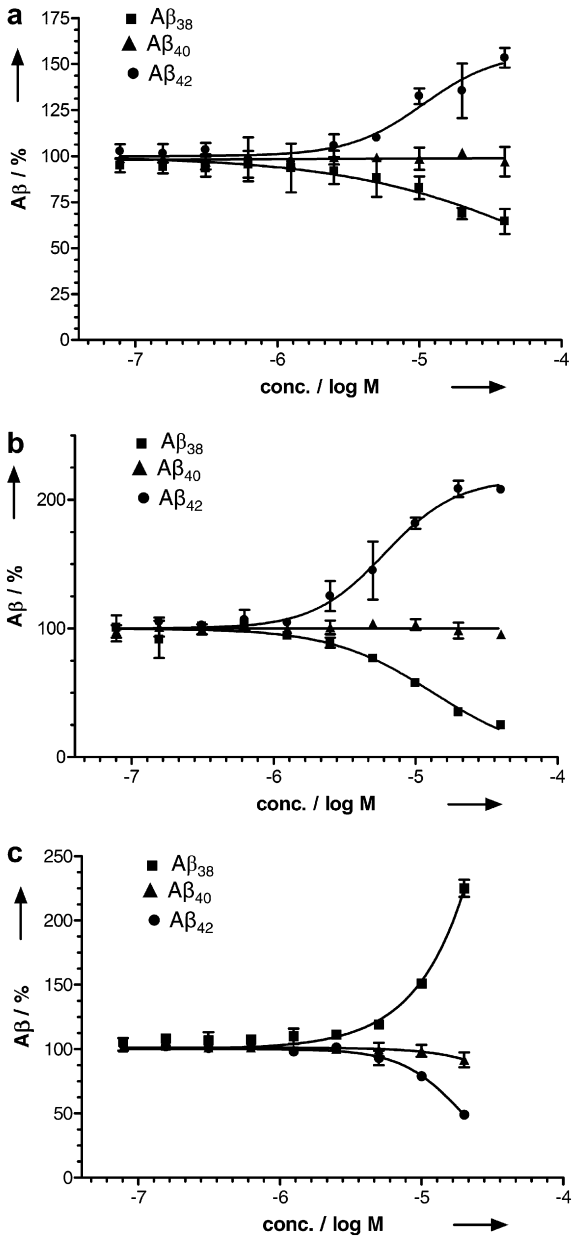
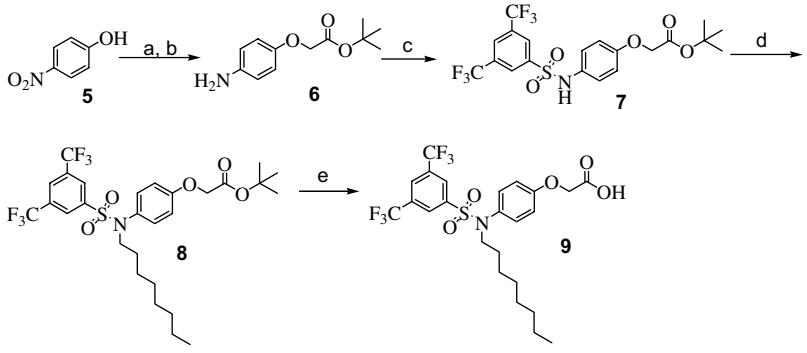


Figure 1. Dose–response curves; Aβ (% of control). (a) Compound **7**. (b) Compound **8**. (c) Compound **9**.

sulfonamide **7**. N-Alkylation of sulfonamide **7** was accomplished using KO^{t}Bu and *n*-octyl iodide in DMF to provide the alkylated product **8**. The subsequent acidic cleavage of *tert*-butyl ester gave acid **9** as colourless solid.

Compounds **7**–**9** were tested for their ability to modulate γ-secretase in the cellular assay; we used the Aβ liquid phase electrochemiluminescence assay to measure secreted Aβ isoforms.[26] In accordance with previous results,[25,26] the esters **7** and **8** (see Table 1 and Fig. 1) affected the cleavage at the γ38, γ40 and γ42 sites as the inverse modulators **2b** and **2c**. They suppressed the formation of $A\beta_{38}$, while enhancing the formation of $A\beta_{42}$. N-Alkylation of the sulfonamide **7** enhanced the inverse γ-secretase modulatory activity of compound **8**. The transformation of the ester **8** into the free acid **9** reversed the inverse modulation to normal modulation. Acid **9** displayed a typical NSAID like profile of secreted Aβ fragments. It particularly reduced the formation of $A\beta_{42}$ and enhanced the formation of the less toxic $A\beta_{38}$. Moreover, it showed toxicity at 40 μM concentration. Acid **9** is likely to have little effect on the γ-secretase cleavage at the ε-site.[25,26] These observations confirm our hypothesis that the free acid moiety is crucial for modulation of PS1. We speculate that the lipophilic substituent anchors the N-substituted phenoxyacetic acid in the required orientation within the membrane, thus the maximum tolerated length should be similar to natural phospholipids (Scheme 2).

Replacement of the hexafluorocarbinol moiety of LXR agonist, **TO-901317**, by an oxyacetic acid group changed the mode of action from inverse modulation to modulation. In accordance with previous results, the derivatisation of the acid into esters such as the compounds **7** and **8** provided inverse modulators. Introduction of *n*-octyl, a lipophilic substituent on the sulfonamide **7**, increased the inverse modulatory activity. Compound **9** with the free acid was found to be a normal modulator of γ-secretase. Again, it is evident from the structure–activity relationship data that the free acid functionality is crucial for the PS modulatory activity. This modification can be applied to several γ-secretase inverse modulators and is a reliable tool to



Scheme 2. Synthesis of the LXR agonist analogous γ-secretase modulator. Reagents and conditions: (a) $ClCH_2CO_2{}^tBu$, K_2CO_3, DMF, rt, 4 h, 97%; (b) 10% Pd–C, H_2, 60 psi, EtOAc, 6 h, rt, 95%; (c) 3,5-bis-(trifluoromethyl) benzene sulfonyl chloride, Et_3N, DMAP, DCM, rt, 12 h, 68%; (d) KO^{t}Bu, *n*-octyl iodide, DMF, rt, 36 h, 50%; (e) 20% TFA in DCM, rt, 6 h, 90%.

convert the mode of action from inverse modulation to modulation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.044.

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